To: Councell, Terry[Terry.Councell@fda.hhs.gov]
Cc: Hrdy, David[Hrdy.David@epa.gov]
From: Miller, David
Sent: Tue 7/28/2015 12:33:07 PM
Subject: RE: glyphosate

Is this for a formal tele- meeting or just a conversation between you and me? I guess I had thought it was the latter. Who from FDA will be attending?

Can you let me know the specific topic/issue. Does it relate to the FDA sampling for glyphosate and whether it is representative or not.

By the way, David H. is out yesterday and today at the NFPA meeting on Orlando. So tomorrow (Wednesday) would have been better anyway.

Please let me know about the topic/attendees and I will mail you back with times that David H. and I are available.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, July 28, 2015 7:15 AM
To: Miller, David
Subject: RE: glyphosate

David

I can't get anyone here at 3:30. Any time available Wednesday?

Terry

From: Miller, David [mailto:Miller.DavidJ@epa.gov]

Sent: Monday, July 27, 2015 7:24 PM
To: Councell, Terry
Subject: RE: glyphosate

Yes, tomorrow will be ok. Maybe 3:30?

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Monday, July 27, 2015 12:46 PM
To: Miller, David
Subject: glyphosate

David

Do you have time for a call tomorrow on glyphosate? Let me know a time

Terry Councell

Total Diet Study Coordinator

Food and Drug Administration

Office of Analytics and Outreach

5100 Paint Branch Parkway

College Park, MD 20740

PH (240) 402 1180

To: Councell, Terry[Terry.Councell@fda.hhs.gov]
From: Miller, David
Sent: Mon 7/27/2015 11:24:14 PM
Subject: RE: glyphosate

Yes, tomorrow will be ok. Maybe 3:30?

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Monday, July 27, 2015 12:46 PM
To: Miller, David
Subject: glyphosate

David

Do you have time for a call tomorrow on glyphosate? Let me know a time

Terry Councell

Total Diet Study Coordinator

Food and Drug Administration

Office of Analytics and Outreach

5100 Paint Branch Parkway

College Park, MD 20740

PH (240) 402 1180

To: Councell, Terry[Terry.Councell@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]
From: Miller, David
Sent: Wed 7/22/2015 1:17:39 PM
Subject: RE: Glyphosate

Hello Terry,

I will be here and available on Monday.

David – could you please send Terry available times.

Tx.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Wednesday, July 22, 2015 8:52 AM
To: Miller, David; Hrdy, David
Subject: Glyphosate

Would you all be available for a call on glyphosate this coming Monday (27th?)? What time would work out for you? I'll get the call set up and call in numbers.

Thanks

Terry Councell

Food and Drug Administration

Office of Food Safety

5100 Paint Branch Parkway

College Park, MD 20740

PH (240) 402 1180

To: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]
Cc: Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Herndon, George
Sent: Thur 4/7/2016 2:34:30 PM
Subject: Pesticides Residues in Honey

Chris and Charlotte,

Sounds like Lauren wants us to meet as a smaller group before something is proposed to the larger group. Let me know if you are ready to have a phone conference call, and if so, what dates/times might work. Thanks!

Jeff

From: Robin, Lauren P [mailto:Lauren.Robin@fda.hhs.gov]
Sent: Thursday, March 24, 2016 10:38 AM
To: Herndon, George <Herndon.George@epa.gov>
Cc: Liang, Charlotte <Charlotte.Liang@fda.hhs.gov>; South, Paul <Paul.South@fda.hhs.gov>; Kashtock, Michael E <Michael.Kashtock@fda.hhs.gov>; Sack, Chris A <Chris.Sack@fda.hhs.gov>; Hrdy, David <Hrdy.David@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff—

Requires Consultation with FDA

Lauren

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Monday, March 21, 2016 5:20 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: RE: Glyphosate Honey Risk Calculations

Robin,

Just following up on the glyphosate slides. Let me know when you want to discuss options for moving forward. Thanks!

Jeff

From: Robin, Lauren P [mailto:Lauren.Robin@fda.hhs.gov]
Sent: Wednesday, March 09, 2016 9:50 AM
To: Herndon, George <Herndon.George@epa.gov>
Cc: Liang, Charlotte <Charlotte.Liang@fda.hhs.gov>; South, Paul <Paul.South@fda.hhs.gov>; Kashtock, Michael E <Michael.Kashtock@fda.hhs.gov>; Sack, Chris A <Chris.Sack@fda.hhs.gov>; Hrdy, David <Hrdy.David@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff—

Requires Consultation with FDA

Regards

Lauren

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 2:01 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

To: Herndon, George[Herndon.George@epa.gov]
Cc: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Robin, Lauren P
Sent: Thur 3/24/2016 2:38:06 PM
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff—

Requires Consultation with FDA

Lauren

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Monday, March 21, 2016 5:20 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: RE: Glyphosate Honey Risk Calculations

Robin,

(b) (5)

Jeff

From: Robin, Lauren P [mailto:Lauren.Robin@fda.hhs.gov]
Sent: Wednesday, March 09, 2016 9:50 AM
To: Herndon, George <Herndon.George@epa.gov>

Cc: Liang, Charlotte <Charlotte.Liang@fda.hhs.gov>; South, Paul <Paul.South@fda.hhs.gov>; Kashtock, Michael E <Michael.Kashtock@fda.hhs.gov>; Sack, Chris A <Chris.Sack@fda.hhs.gov>; Hrdy, David <Hrdy.David@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff—

Requires Consultation with FDA

Regards

Lauren

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 2:01 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

To: Robin, Lauren P[Lauren.Robin@fda.hhs.gov]
Cc: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Herndon, George
Sent: Mon 3/21/2016 9:20:12 PM
Subject: RE: Glyphosate Honey Risk Calculations

Robin,

(b) (5)

Jeff

From: Robin, Lauren P [mailto:Lauren.Robin@fda.hhs.gov]
Sent: Wednesday, March 09, 2016 9:50 AM
To: Herndon, George <Herndon.George@epa.gov>
Cc: Liang, Charlotte <Charlotte.Liang@fda.hhs.gov>; South, Paul <Paul.South@fda.hhs.gov>; Kashtock, Michael E <Michael.Kashtock@fda.hhs.gov>; Sack, Chris A <Chris.Sack@fda.hhs.gov>; Hrdy, David <Hrdy.David@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff—

Requires Consultation with FDA

Regards

Lauren

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 2:01 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

To: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]
Cc: Robin, Lauren P[Lauren.Robin@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Miller, David[Miller.DavidJ@epa.gov]; Hrdy, David[Hrdy.David@epa.gov]
From: Herndon, George
Sent: Fri 3/11/2016 4:31:35 PM
Subject: RE: Glyphosate Honey Risk Calculations

Charlotte,

(b) (5)

Jeff

From: Liang, Charlotte [mailto:Charlotte.Liang@fda.hhs.gov]
Sent: Friday, March 11, 2016 11:15 AM
To: Herndon, George <Herndon.George@epa.gov>
Cc: Robin, Lauren P <Lauren.Robin@fda.hhs.gov>; Sack, Chris A <Chris.Sack@fda.hhs.gov>
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff:

Requires Consultation with FDA

Thanks,

Charlotte

Charlotte Liang, Ph.D.

Plant Products Branch

Division of Plant Products and Beverages

Office of Food Safety

Center for Food Safety and Applied Nutrition

Food and Drug Administration

Phone:240-402-2785

Fax:301-436-2632

charlotte.liang@fda.hhs.gov

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 2:01 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

To: Herndon, George[Herndon.George@epa.gov]
Cc: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Robin, Lauren P
Sent: Wed 3/9/2016 2:50:27 PM
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff—

Requires Consultation with FDA

Regards

Lauren

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 2:01 PM
To: Robin, Lauren P
Cc: Liang, Charlotte; South, Paul; Kashtock, Michael E; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

To: Robin, Lauren P[Lauren.Robin@fda.hhs.gov]
Cc: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Herndon, George
Sent: Thur 3/3/2016 7:00:33 PM
Subject: Glyphosate Honey Risk Calculations
EPA Screening Assessment on Glyphosate in Honey2 23 16.pptx

Lauren,

(b) (5)

Jeff

(b) (5)

(b) (5)

(b) (5)

To: Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; Robin, Lauren P[Lauren.Robin@fda.hhs.gov]
Cc: South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Herndon, George
Sent: Thur 3/3/2016 3:21:35 PM
Subject: RE: Glyphosate Honey Risk Calculations

Charlotte,

(b) (5)

Jeff

From: Liang, Charlotte [mailto:Charlotte.Liang@fda.hhs.gov]
Sent: Thursday, March 03, 2016 9:53 AM
To: Herndon, George <Herndon.George@epa.gov>; Robin, Lauren P <Lauren.Robin@fda.hhs.gov>
Cc: South, Paul <Paul.South@fda.hhs.gov>; Kashtock, Michael E <Michael.Kashtock@fda.hhs.gov>; Sack, Chris A <Chris.Sack@fda.hhs.gov>; Hrdy, David <Hrdy.David@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate Honey Risk Calculations

Hi Jeff:

Requires Consultation with FDA

Thanks.

Charlotte

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 9:27 AM
To: Robin, Lauren P
Cc: South, Paul; Kashtock, Michael E; Liang, Charlotte; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

To: Herndon, George[Herndon.George@epa.gov]; Robin, Lauren P[Lauren.Robin@fda.hhs.gov]
Cc: South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Liang, Charlotte
Sent: Thur 3/3/2016 2:53:00 PM
Subject: RE: Glyphosate Honey Risk Calculations
survey-of-glyphosate-residues-in-honey-corn-and-soy-products-2161-0525.1000249 (1).pdf

Hi Jeff:

Requires Consultation with FDA

Thanks.

Charlotte

From: Herndon, George [mailto:Herndon.George@epa.gov]
Sent: Thursday, March 03, 2016 9:27 AM
To: Robin, Lauren P
Cc: South, Paul; Kashtock, Michael E; Liang, Charlotte; Sack, Chris A; Hrdy, David; Miller, David
Subject: Glyphosate Honey Risk Calculations

Lauren,

(b) (5)

Jeff

Environmental & Analytical Toxicology

Rubio et al., J Environ Anal Toxicol 2014, 5:1
http://dx.doi.org/10.4172/2161-0525.1000249

Research Article Open Access

Survey of Glyphosate Residues in Honey, Corn and Soy Products

Fernando Rubio[1*], Emily Guo[2] and Lisa kamp[1]

[1]*Abraxis, LLC, 54 Steam whistle Drive, Warminster, PA 18974, USA*
[2]*Boston University, 273 Babcock Street, Boston, MA 02446, USA*

Abstract

Samples of honey (sixty nine), pancake and corn syrup (twenty six), soy sauce (twenty eight), soy milk (eleven), and tofu (twenty) purchased in the Philadelphia, US metropolitan area were analyzed for glyphosate residue using ELISA. The limit of quantification (LOQ) and range of the method were determined for honey, pancake syrup, and corn syrup to be 15 to 800 ppb; soy sauce, soy milk, and tofu 75 to 4,000 ppb. Glyphosate residues above the limit of quantification were not found in pancake and corn syrup, soy milk, and tofu. Of the sixty-nine honey samples analyzed, forty-one samples, or fifty-nine percent (59%), had glyphosate concentrations above the method LOQ (15 ppb), with a concentration range between 17 and 163 ppb and a mean of 64 ppb. Eleven of the tested honey samples were organic; five of the organic honey samples, or forty-five percent (45%), contained glyphosate concentrations above the method LOQ, with a range of 26 to 93 ppb and a mean of 50 ppb. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62%), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb. In addition to comparison of production method (organic vs. conventional), the honey results were evaluated according to pollen source and by country of origin, grouped by GMO usage (prohibited, limited, or permitted). Glyphosate concentrations above the method LOQ (75 ppb) were also found in ten of the twenty-eight soy sauce samples evaluated (36%), with a concentration range between 88 and 564 ppb and a mean of 242 ppb; all organic soy sauce samples tested were below the method LOQ.

Keywords: Glyphosate; Honey; Honey bees; Soy sauce; ELISA

Introduction

Food consumption is an important pathway of human exposure to pesticides and other chemical contaminants. Studies have shown that exposure to contaminants in food could pose a public health risk [1,2,3]. Contaminants can enter the food supply in various ways including direct pesticide application to food crops, indirect application through the air (from drift from aerial spraying of adjacent fields), through the soil (from direct application during previous growing seasons), through the water supply (from run-off from treated areas), or through food processing (from cross-contamination from shared processing equipment) [4,5].

Glyphosate (N-(phosphonomethyl) glycine) (Figure 1), commonly sold under Monsanto's trade name Roundup®, is a non-selective herbicide that inhibits 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) in the shikimic acid pathway present in plants, bacteria, and archae [6]. EPSP synthase is the rate limiting step in the synthesis of various aromatic acids; inhibition of this enzyme results in depletion of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan [7]. Glyphosate trans locates readily in plants, making it effective for controlling perennial weeds and overwintering rhizomes and tubers. It is registered for pre planting or postharvest treatment on crops and on non-crop land [8]. Although humans do not posses the shikimic acid pathway, we are dependent upon ingested food and gut microbes, which provide essential nutrients, which do possess this pathway. Glyphosate has been patented as an antimicrobial by Monsanto Technology LLC [9], and has been shown to disrupt gut bacteria in animals [10-12]. In humans, only a small amount (~2%) of ingested glyphosate is metabolized to amino methyl phosphonic acid (AMPA), the rest enters the blood stream and is eventually eliminated through the urine [13].

The use of glyphosate in agriculture has increased significantly with the introduction of transgenic crops such as Roundup-Ready® soybeans and corn, which enable farmers to directly apply low cost broad spectrum herbicide products to their fields without harming crops. In the United States, glyphosate is currently the most widely used herbicide, with 180 to 185 millions pounds applied in the agricultural sector during 2007, 5 to 8 million pounds used in homes and gardens, and 13-15 million pounds used in industrial, commercial and governmental weed control applications [13]. The dramatic increase in the use of glyphosate in agriculture and landscape maintenance is occurring not only in the US, but throughout the world. This high level of use has led to concerns about its effects on humans and the environment. Glyphosate has traditionally been considered to be nearly non-toxic to humans [14], and therefore not problematic if ingested in food sources; as a consequence, measurement of its presence in food is very scarce [15,16]. Challenge the assertion that glyphosate is harmless, arguing that this herbicide may be a key contributor to the obesity and autism epidemics in the United States, as well as a factor in several diseases and conditions including celiac disease, Alzheimer's, Parkinson's, infertility, depression, and cancer.

Glyphosate analysis in environmental and biological matrices is problematic because of its small molecular size and structural similarity to many naturally occurring plant materials such as amino acids and secondary plant compounds. It is highly soluble in water, thereby making its extraction with solvents difficult and matrix effects highly prevalent. As a result, glyphosate isolation and quantification has posed a challenge to the analytical chemist. Numerous analytical procedures have been published in the literature for the detection of this highly

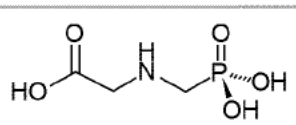

Figure 1: Glyphosate (N-(phosphonomethyl) glycine)

***Corresponding author:** Abraxis, LLC, 54 Steam whistle Drive, Warminster, PA 18974, USA, Tel: (215) 357-3911; FAX: (215) 357-5232; E-mail: frubio@abraxiskits.com

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Citation: Rubio F, Guo E, Kamp L (2014) Survey of Glyphosate Residues in Honey, Corn and Soy Products. J Environ Anal Toxicol 4: 249. doi: 10.4172/2161-0525.1000249

polar and amphoteric molecule [17], including gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), often coupled with mass spectrometry (MS). The co-contaminants in environmental and biological matrixes render instrumental analysis costly and time-consuming. ELISA determination, however, has allowed for the rapid, selective and sensitive determination of glyphosate [18-24].

The main objective of this survey was to obtain representative data on levels of glyphosate residues in honey, corn and pancake syrup, and soy based products, such as soy sauce, soy milk, and tofu, in products consumed by the general population in and around Philadelphia, Pennsylvania. The analytical program included the extraction of glyphosate from the various matrices and the subsequent determination of glyphosate residues by enzyme linked immune sorbent assay (ELISA).

Materials and Methods

Chemicals and reagents

Chemicals were of reagent grade and were purchased from Sigma Chemical Company, St. Louis MO, USA, except as indicated. Glyphosate (>98% purity), Chem Service, West Chester, PA, USA. Glyphosate micro titer plate ELISA, Abraxis PN 500086; Glyphosate sample diluent, PN 500082, Abraxis LLC, Warminster, PA, USA. Glyphosate stock solution was prepared in deionized water to 1.0 mg/mL; spiking solutions were prepared from the working solution using deionized water.

Samples and sample preparation/extraction

In total, 153 representative samples were purchased from markets in the Philadelphia metropolitan area (69 honey, 26 corn and pancake syrup, 28 soy sauce, 11 soy milk, and 20 tofu products).

Honey, corn and pancake syrup samples: A 0.50 g aliquot of sample was weighed into a micro centrifuge tube and 0.50 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:200 sample dilution.

Soy sauce: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Soy milk: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Tofu: A 1.0 g aliquot of sample was weighed into a 20 mL vial and 10.0 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then allowed to separate for 2 minutes. Approximately 1 mL of the mixture was transferred into a micro centrifuge tube and centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Determination of glyphosate in samples

The instructions provided in the ELISA kit user's guide were followed, in brief, glyphosate calibrators provided in the kit and the samples to be tested are derivatized for ten minutes and then added, along with an antibody specific for glyphosate to micro titer wells coated with goat anti-rabbit antibody and incubated for thirty minutes with shaking. A glyphosate horseradish peroxidase (HRP) enzyme conjugate is then added. At this point a competitive reaction occurs between the glyphosate, in the calibrators or samples, and the enzyme labeled glyphosate for the antibody binding sites on the micro titer well. The reaction is allowed to continue for sixty minutes. After a washing step an enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine) are added. The enzyme-labeled glyphosate bound to the glyphosate antibody catalyzes the conversion of the substrate /chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of diluted acid and read in a Molecular Devices micro titer plate reader (450 nm). Since the labeled glyphosate (conjugate) was in competition with the unlabeled glyphosate (sample) for the antibody sites, the color developed is inversely proportional to the concentration of glyphosate in the sample.

Data analysis

The evaluation of the assay was performed using Molecular Devices Softmax pro evaluation program (4-Parameter). The program calculates the mean absorbance value for each of the standards (B_i) and calculates the $\%B_i/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance (B_0). The program then constructs a non-linear regression model of a standard curve by plotting the $\%B_i/B_0$ for each standard on the vertical linear (y) axis versus the corresponding glyphosate concentration on the horizontal logarithmic (x) axis. The $\%B_i/B_0$ for samples is interpolated using the standard curve yielding sample concentration levels of glyphosate from the standard curve. Correlation coefficients of the assays were >0.995 and standard deviation between standard replicate analysis were < 10%.

Validation, performance and quality control

Specificity had been previously determined (ELISA user's guide), (Table 1). Recovery, limit of quantitation, range and limit of quantification were determined to test the validity of the dilution/extraction procedures of each of the matrices used in combination with the glyphosate ELISA.

COMPOUND (B/Bo)	LOD (ng/mL)	50% B/Bo (ng/mL)
Glyphosate	0.05	0.5
Glyphosine	50	3,000
Glufosinate	2,000	70,000
AMPA	35,000	>1,000,000
Glycine	>10,000	>1,000,000

Table 1: Cross-reactivity table. The reactivity of glyphosate to various related compounds expressed as LOD and as the dose required for 50% absorbance inhibition (50% B/Bo).

Results and Discussion

The method performance for glyphosate analysis was determined by conducting recovery tests on each of the matrices. To determine the accuracy of the glyphosate analysis for the sample matrices analyzed in this study, matrix samples that were glyphosate negative and positive (positive samples were not encountered with tofu, soy milk, pancake and corn syrup) were spiked as follows: 15, 40, 100, 200 and 400 ng/mL (honey, pancake and corn syrup); 75, 200, 500, 1,000 and 4,000 ng/mL [soy sauce, soy milk and tofu (ng/g)]. Analysis was performed in duplicate for all unspiked and spiked samples at all levels. Average recovery obtained for glyphosate negative honey samples fortified with glyphosate was 119 %, (SD = 10). Average recovery for glyphosate positive honey (unspiked contained 44 ng/g glyphosate) after fortification was 116 % (SD = 10). Average recovery for negative soy sauce was 94% (SD = 5), and for positive fortified soy sauce (unspiked contained 417 ng/mL) was 86% (SD = 5). The limit of quantification and range of the method were determined for honey, pancake and corn syrup to be 15 to 800 ng/g; soy sauce, soy milk, and tofu 75 to 4,000 ng/mL or ng/g, respectively.

In this study, the first sample matrix analyzed for the presence of glyphosate was honey; 69 samples were analyzed and classified into 18 groups depending on the country of origin listed on the bottles: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. The glyphosate concentrations obtained are shown in (Figure 2). Forty-one out of the sixty-nine honey samples analyzed, or fifty nine percent (59 %), had glyphosate concentrations above the method LOQ (15 ng/g) with a concentration range between 17 and 163 ng/g and a mean of 64 ng/g.

The glyphosate concentration in honey grouped by flower (pollen) source is shown in (Figure 3). The pollen types listed on the bottles were: clover (12 samples), exotic (11 samples), wildflower (11 samples), unknown (35 samples). (Figure 4) depicts the concentration of glyphosate in honey samples grouped by growing method of source pollen: organic (11 samples) and traditional (58 samples); 5 of the 11 organic samples had glyphosate concentrations above the method LOQ with a range of 26 to 93 ng/g and a mean of 50 ng /g. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62%), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb.

(Figure 5) depicts the concentration of glyphosate in honey by country and whether the use of genetically modified organisms (GMO) seeds is prohibited or permitted. The graph also shows where some minimum uses of GMO traits are allowed (Spain, and blend of Vietnam/Ukraine). The glyphosate concentration in honey originating in countries that do not allow or allow limited GMO traits (3 out of 14 samples above the LOQ) ranged from 26 to 41 ng/g with a mean of 31 ng /g. The glyphosate range for those countries that allow GMO (30 out of 43 samples above LOQ) was 21 to 163 ng/g with a mean of 71 ng /g. Samples of unknown origin (8 out of 12 samples above LOQ) ranged from 17 to 95 ng/g with a mean of 50 ng/g.

The second matrix group analyzed for glyphosate was soy sauce. The analysis consisted of 28 samples, (Figure 6). Ten out of 28 samples (36 %) had glyphosate concentrations above the method LOQ (75 ng/

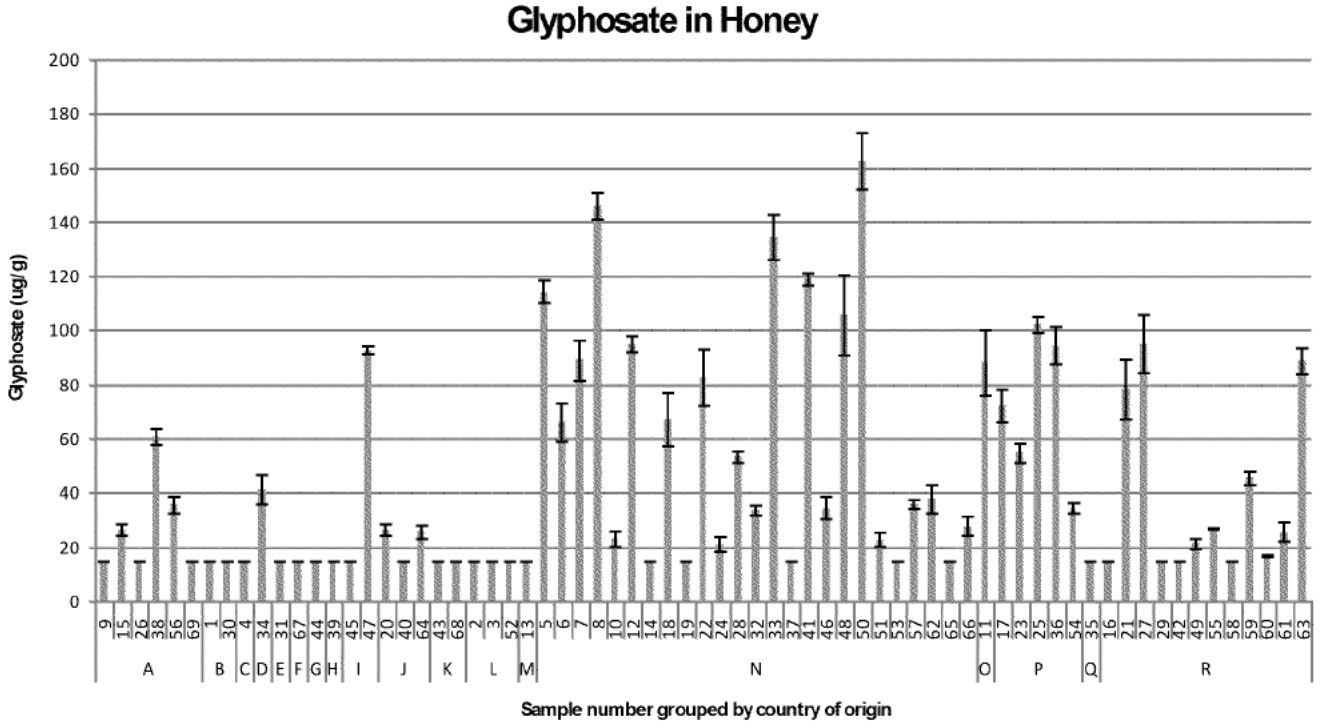

Figure 2: Concentration of glyphosate (ng/g) in honey samples listed by honey origin: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g). Error bars represent concentrations obtained during duplicate analysis.

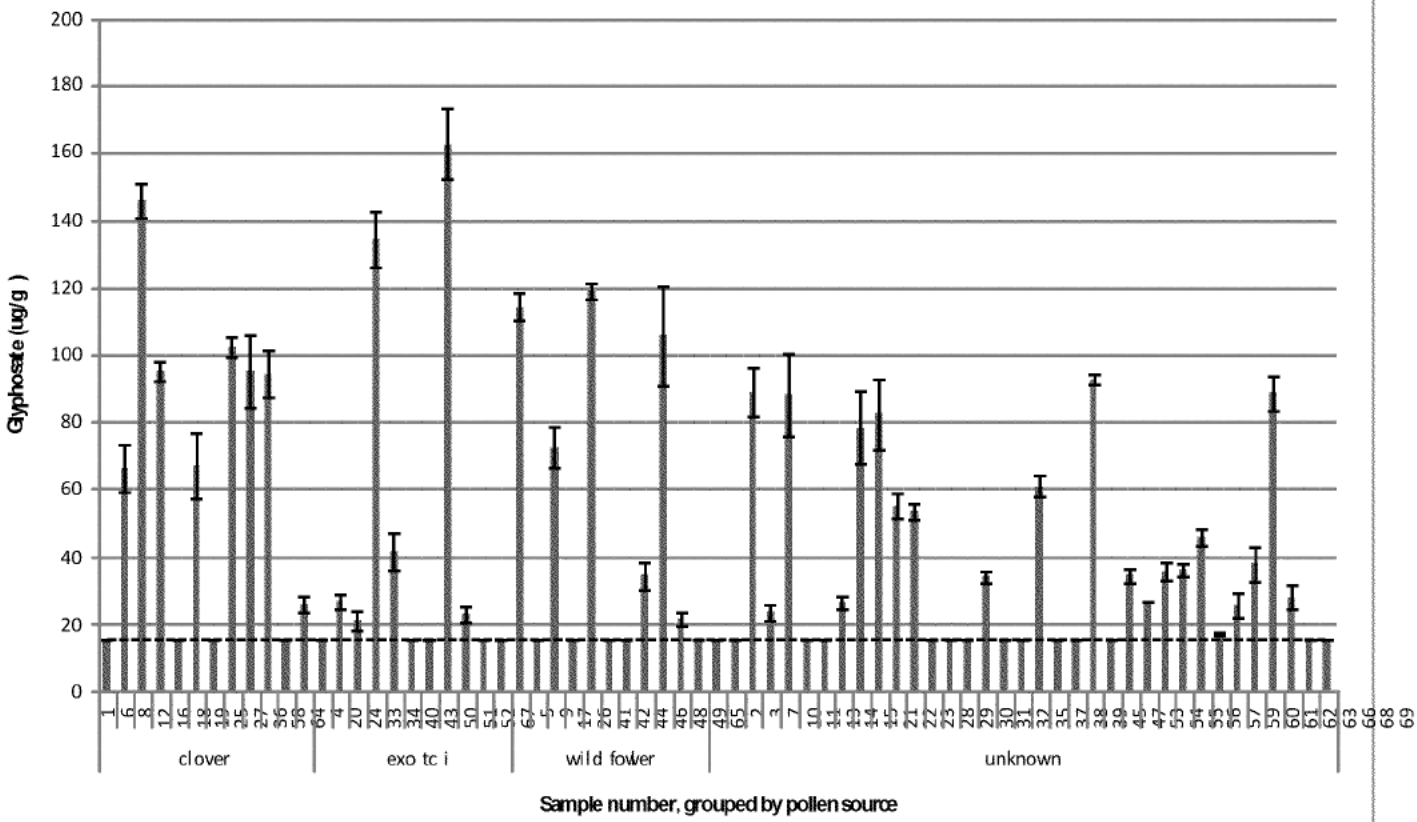

Figure 3: Concentration of glyphosate (ng/g) in honey samples by flower (pollen) source. Dashed line represents LOQ of method (15 ng/g). Exotic flowers were sophora, manuka, orange, cactus, summer flower, lychee, alfalfa, acacia.). Error bars represent concentrations obtained during duplicate analysis.

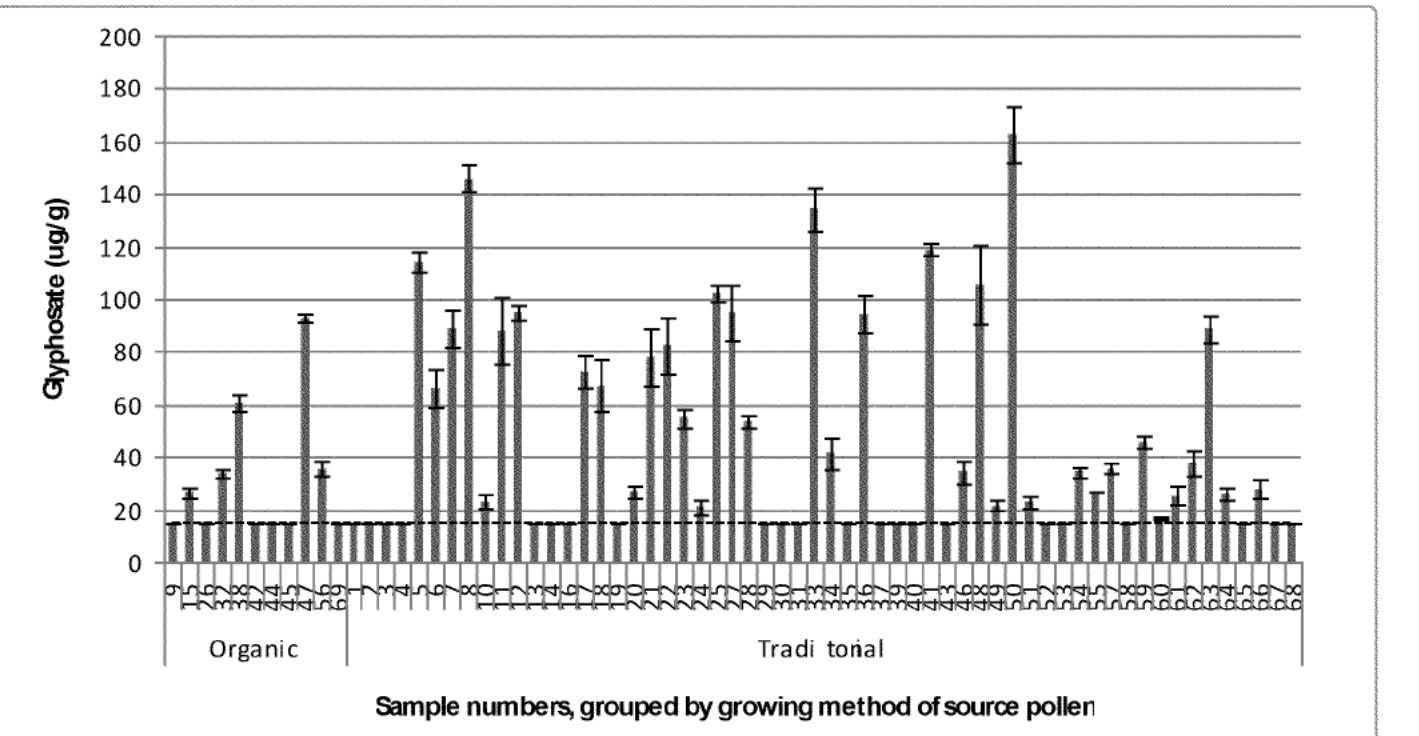

Figure 4: Concentration of glyphosate (ng/g) in honey samples by growing method of source pollen (Organic vs.Traditional). Dashed line represents LOQ of method (15 ng/g).) Error bars represent concentrations obtained during duplicate analysis.

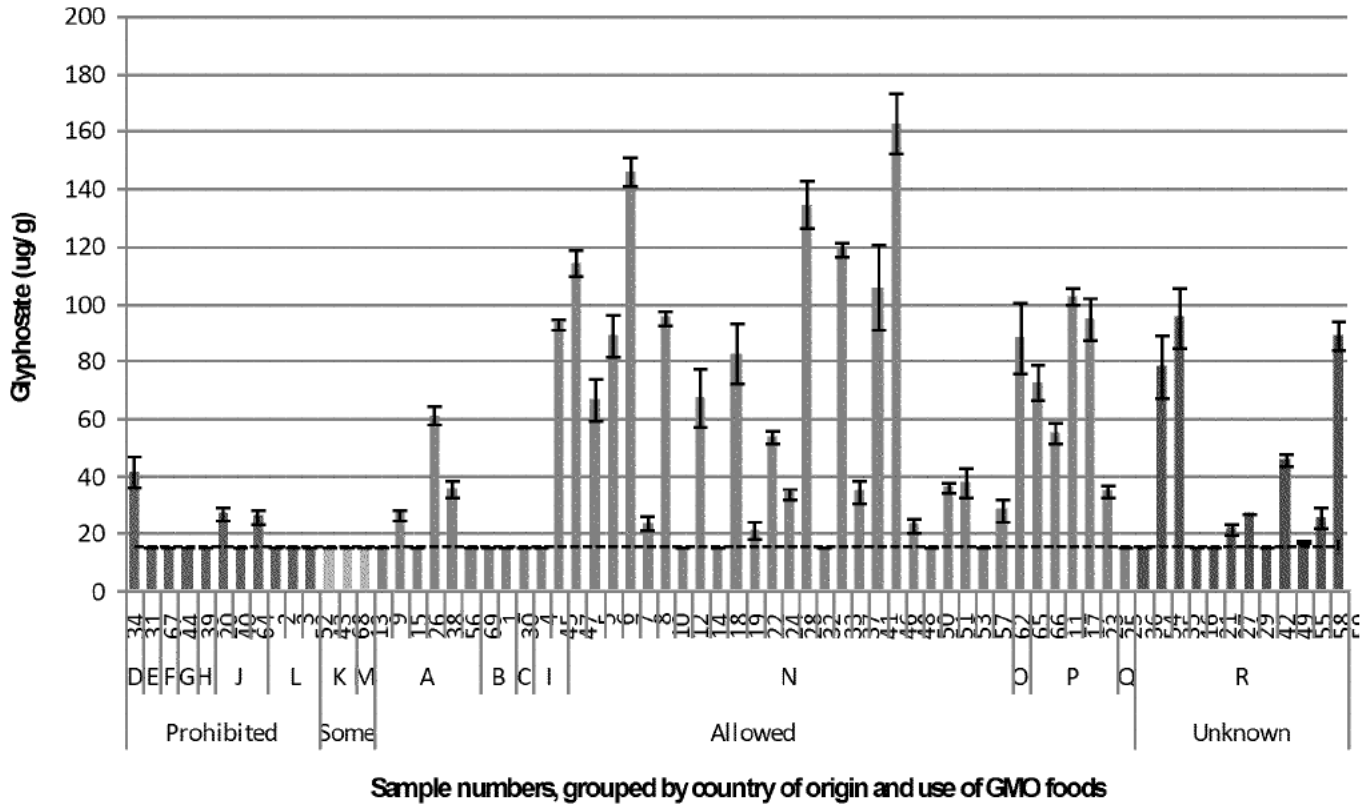

Figure 5: Concentration of glyphosate (ng/g) in honey samples listed by honey origin and the allowance of GMO use: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g).). Error bars represent concentrations obtained during duplicate analysis.

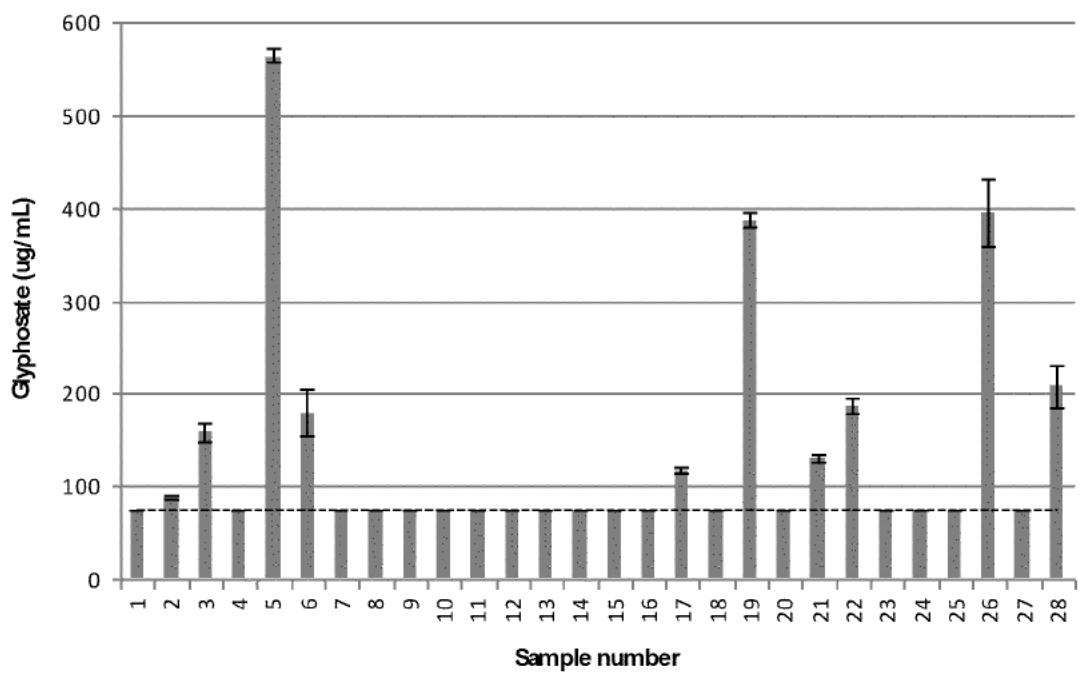

Figure 6: Concentration of glyphosate (ng/mL) in soy sauce samples. Dashed line represents LOQ of method (75 ng/mL).). Error bars represent concentrations obtained during duplicate analysis.

mL) with a concentration range between 88 and 564 ng/mL and a mean of 242 ng /mL. (Figure 7) shows the concentration of glyphosate in soy sauce by method of soy bean growing (organic vs. traditional). Therecent report from the Chinese Academy of Medical Science and the Beijing Union Hospital [20] reported an average glyphosate concentration in soy sauce of 133 ng/mL in samples that did not specify on the bottle whether or not the raw material was GM soybean. In our study, the small subset of organic labeled samples (three) was all below the limit of quantitation of the test.

Corn and pancake syrup (26 samples), soy milk (11 samples), and tofu (20 samples) tested were negative for glyphosate at the LOQ of the method (15 ng/g for pancake and corn syrup, and 75 ng/mL or ng/g for soy milk and tofu, respectively).

Studies on glyphosate residues in food are scarce. Among the few studies found was a recent report published on the incidence of glyphosate in soy sauce, conducted by the Chinese government [20]. Searches were conducted by the authors using various scientific databases on the concentration and incidence of glyphosate in honey, but these failed to provide any information. Thehoney samples analyzed in the present study show that 59 % of all samples contained glyphosate residues (ranging from 17 to 163 ng/g, mean 64 ng/g); the residue concentration does not seem to depend on pollen source or growing method, even organic honey contained glyphosate residues (5 out 11 samples, or 45 %, mean glyphosate concentration 50 ng/g). Comparing the concentration of glyphosate in honey by countries that use GMO extensively with countries that allow the use of some GMO traits and those that do not allow GMO, shows that, in general, glyphosate levels are lower in samples from countries that do not allow or allow limited use of some GMO traits, such as Spain and Vietnam/ Ukraine blend (mean 31 ng/g), compared to those countries that allow planting of GMO traits (71 ng/g). It should be noted, however, that some residues of glyphosate (although < 50 ng/g) were found in honeys originating from Germany and New Zealand, countries where no GMO planting is allowed.

TheEuropean Union has specificguidelines for the labeling of organic honey [25,26]. According to those guidelines, the location of apiaries is strictly controlled and states that "Nectar and pollen sources available over a three-kilometer radius around the apiary sites must consist essentially of organically produced crops or crops treated with low-environmental-impact methods. Apiaries must also be far enough away from any non-agricultural production source that could lead to contamination (e.g. urban centers, waste dumps, waste incinerators, etc.). Member States have the option of prohibiting the production of organic honey in certain regions or areas that do not meet these conditions. Organic honey must not contain chemicals residues (synthetic pesticides, etc.)." TheUnited States has no such guidelines for the organic production of honey, but uses organic farming certification for honey labeling purposes; one reason is that it is practically impossible to regulate without testing all honey for residues since bees can flyup to 3 miles in search of nectar and it is difficult to be certain that they do not feed on nectar contaminated by crop spraying or industrial sources. In the EU, glyphosate residues in non-organic honey regulatory limits are 50 ng/g [27], the United States does not have a limit in honey. Thelimit in drinking water in the United States is 700 ng/mL; the reference dose is 1.75 mg/Kg/day; the One-Day Health Advisory level is 20 mg/L [28]. Also, it is widely known that like milk and olive oil, honey is one of the foods that is most commonly mislabeled and adulterated [29] providing yet another source of glyphosate contamination in honeys that, according to the bottle label, originated in non-GMO countries.

Bee colony collapse disorder (CCD) is a growing threat to the efficient production of food around the world. Honey bees pollinate nearly 130 species of plant life [30], such as fruits, vegetables, nuts, and seed crops. Honeybees are therefore indirectly responsible for an

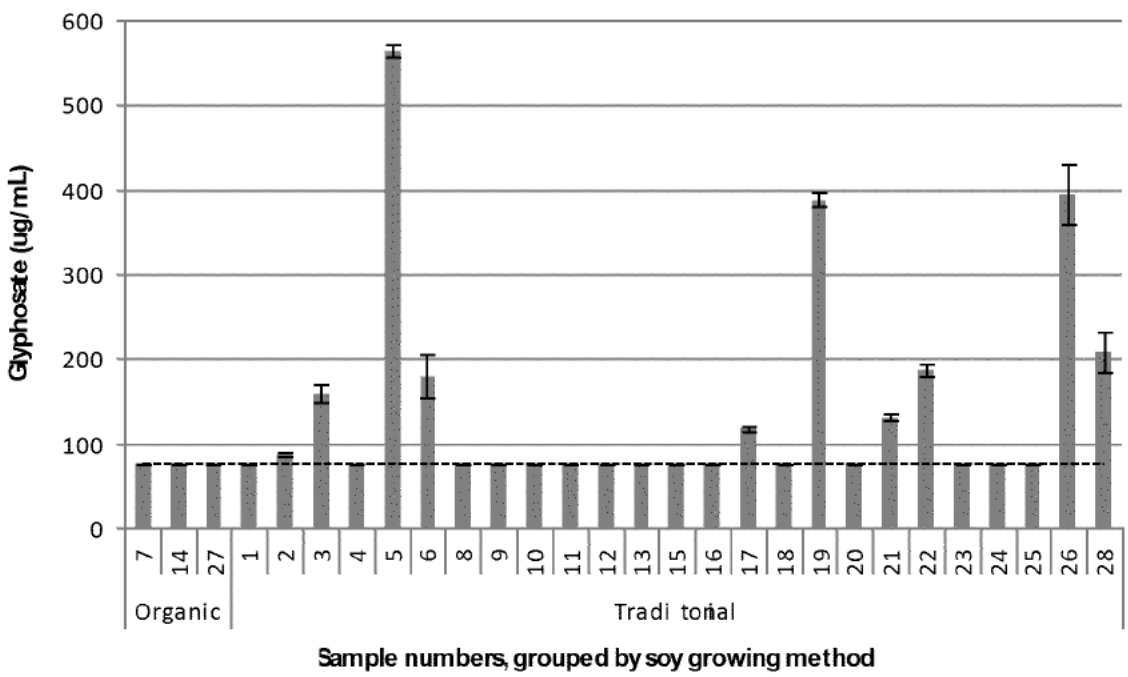

Figure 7: Concentration of glyphosate (ng/mL) in soy sauce samples by growing method of soy beans (Organic vs.Traditional). Dashed line represents LOQ of method (75 ng/mL).). Error bars represent concentrations obtained during duplicate analysis.

estimated one-third of the world food supply [31]. Although several factors are involved in CCD, including numerous pathogens and parasites, the extensive use of pesticides [32,33] such as neonicotinoids have provided evidence that these products are harmful to honey bees and have lead to a recent ban or restriction in the use of three neonicotoids by the European Union [34]. Although glyphosate is not acutely toxic to bees, it is chronically toxic to animals and is reported to disrupt the endocrine system [35,36] and a recent study indicates that honey bees exposed to increasing sub-lethal concentrations of glyphosate exhibit a decrease in acetyl cholinesterase (AChE) activity [37]. Thehigh rate of glyphosate use creates the potential for wide-spread contamination of our food chain. Glyphosate is used throughout the bee foraging period in high amounts and is found in the air, water, and in plant parts frequented by bees, such as flowers and buds, potentially contaminating the nectar collected by bees from contaminated plants [38]. Based on its prevalence in the environment, as well as our findingsin honey samples, we propose that future studies should be conducted to determine if glyphosate is in fact a contributing factor in CCD.

Conclusion

Thistudy indicates the presence of glyphosate residues in honey and soy sauce, but not in pancake and corn syrups or soy based products such as soy milk and tofu. Forty one out of sixty nine (59%) honey samples analyzed contained glyphosate at a concentration above the method LOQ (15 ng/g) with a range between 17-163 ng/g and a mean of 64 ng/g. Ten out of twenty eight (36%) soy sauce samples contained glyphosate at a concentration above the method LOQ (75 ng/mL) with a range between 88-564 ng /mL and a mean of 242 ng /mL. Future studies should be conducted on many other food products to determine the extent of glyphosate residue contamination.

Acknowledgements

We would like to give our thanks to Erin Faltin for the preparation of the graphs presented in this paper.

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Citation: Rubio F, Guo E, Kamp L (2014) Survey of Glyphosate Residues in Honey, Corn and Soy Products. J Environ Anal Toxicol 4: 249. doi: 10.4172/2161-0525.1000249

To: Robin, Lauren P[Lauren.Robin@fda.hhs.gov]
Cc: South, Paul[Paul.South@fda.hhs.gov]; Kashtock, Michael E[Michael.Kashtock@fda.hhs.gov]; Liang, Charlotte[Charlotte.Liang@fda.hhs.gov]; Sack, Chris A[Chris.Sack@fda.hhs.gov]; Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Herndon, George
Sent: Thur 3/3/2016 2:26:44 PM
Subject: Glyphosate Honey Risk Calculations
EPA Screening Assessment on Glyphosate in Honey2 23 16.pptx

Lauren,

(b) (5)

Jeff

(b) (5)

(b) (5)

(b) (5)

To: (Chris.Sack@fda.hhs.gov)[Chris.Sack@fda.hhs.gov]
Cc: mike.papathakis@cdpr.ca.gov[mike.papathakis@cdpr.ca.gov]; Miller, David[Miller.DavidJ@epa.gov]; Crowley, Matthew[Crowley.Matthew@epa.gov]; Vogel, Dana[Vogel.Dana@epa.gov]; Smith, Charles[Smith.Charles@epa.gov]; Bloem, Thomas[Bloem.Thomas@epa.gov]
From: Hrdy, David
Sent: Thur 3/3/2016 1:43:16 PM
Subject: FW: Glyphosate tolerance questions

Hi Chris,

(b) (5)

David

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David E. Hrdy
Senior Scientist
CEB/HED/OPP/OCSPP
US EPA www.epa.gov/pesticides
Mailcode 7509P
Telephone: **703.305.6990**
Fax: 703.305.5147
OFFICE 10248 Potomac Yard 1 (South)

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From: Bloem, Thomas
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Sent: Thursday, March 03, 2016 7:07 AM
To: Hrdy, David <Hrdy.David@epa.gov>
Subject: RE: Glyphosate tolerance questions

That is correct AMPA is not in the tolerance expression.

AMPA used to be in the tolerance expression but was eliminated in the early 90s based on tox considerations.

From: Hrdy, David
Sent: Wednesday, March 02, 2016 4:27 PM
To: Bloem, Thomas <Bloem.Thomas@epa.gov>
Subject: FW: Glyphosate tolerance questions

Tom,

Just confirming – no AMPA, right?

David

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David E. Hrdy
Senior Scientist
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From: Sack, Chris A [mailto:Chris.Sack@fda.hhs.gov]
Sent: Wednesday, March 02, 2016 3:15 PM
To: Hrdy, David <Hrdy.David@epa.gov>
Cc: mike.papathakis@cdpr.ca.gov
Subject: Glyphosate tolerance questions

Hi David,

Requires Consultation with FDA

Thanks,

Chris Sack

Residue Expert

Office of Food Safety

Center for Food Safety and Applied Nutrition

US Food and Drug Administration

Phone: 240-402-2464

To: Miller, David[Miller.DavidJ@epa.gov]
From: Councell, Terry
Sent: Tue 1/5/2016 5:19:51 PM
Subject: RE: Glyphosate

Requires Consultation with FDA

From: Miller, David [mailto:Miller.DavidJ@epa.gov]
Sent: Tuesday, January 05, 2016 12:18 PM
To: Councell, Terry
Subject: RE: Glyphosate

(b) (5)

david

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, January 05, 2016 12:15 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate

Requires Consultation with FDA

Terry

From: Miller, David [mailto:Miller.DavidJ@epa.gov]
Sent: Tuesday, January 05, 2016 12:13 PM
To: Councell, Terry
Subject: RE: Glyphosate

(b) (5)

David.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, January 05, 2016 12:02 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: Glyphosate

David

Requires Consultation with FDA

Terry

To: Miller, David[Miller.DavidJ@epa.gov]
From: Councell, Terry
Sent: Tue 1/5/2016 5:14:31 PM
Subject: RE: Glyphosate

Requires Consultation with FDA

Terry

From: Miller, David [mailto:Miller.DavidJ@epa.gov]
Sent: Tuesday, January 05, 2016 12:13 PM
To: Councell, Terry
Subject: RE: Glyphosate

(b) (5)

David.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, January 05, 2016 12:02 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: Glyphosate

David

Requires Consultation with FDA

Terry

To: Miller, David[Miller.DavidJ@epa.gov]
From: Councell, Terry
Sent: Tue 1/5/2016 5:01:42 PM
Subject: Glyphosate

David

Requires Consultation with FDA

Terry

To: Miller, David[Miller.DavidJ@epa.gov]; Hrdy, David[Hrdy.David@epa.gov]
From: Councell, Terry
Sent: Fri 12/4/2015 9:08:46 PM
Subject: FW: total diet data

Requires Consultation with FDA

Terry

From: Chamkasem, Narong
Sent: Friday, December 04, 2015 3:59 PM
To: Councell, Terry
Subject: total diet data

Hi Terry:

Requires Consultation with FDA

Requires Consultation with FDA

Narong Chamkasem, Ph.D.
Southeast Regional Laboratory
Chemistry II/ Pesticides
Phone (404) 253 – 2302
Fax 404-253-1209
narong.chamkasem@fda.hhs.gov

Requires Consultation with FDA

To: Councell, Terry[Terry.Councell@fda.hhs.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Hrdy, David
Sent: Thur 11/12/2015 9:55:29 PM
Subject: RE: Here is the Journal article on glyphosate the news article was based on

Thanks Terry.

><((((º>`·.¸¸.·´¯`·...¸><((((º> ><((((º>.¸.·´¯`·...¸><((((º>¸.

David E. Hrdy
Senior Scientist
CEB/HED/OPP/OCSPP
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OFFICE 10248 Potomac Yard 1 (South)
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From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Thursday, November 12, 2015 8:32 AM
To: Hrdy, David <Hrdy.David@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>
Subject: Here is the Journal article on glyphosate the news article was based on

FYI

Terry Councell

Total Diet Study Coordinator

Food and Drug Administration

Office of Analytics and Outreach

5100 Paint Branch Parkway

College Park, MD 20740

PH (240) 402 1180
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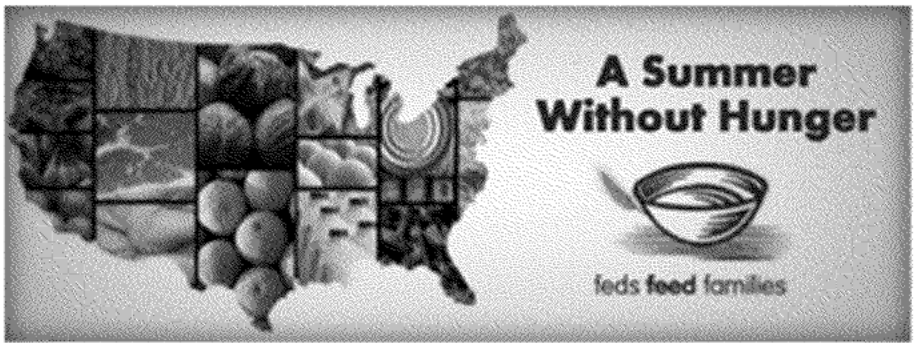
To: Miller, David[Miller.DavidJ@epa.gov]; (Chris.Sack@fda.hhs.gov)[Chris.Sack@fda.hhs.gov]
From: Hrdy, David
Sent: Thur 11/12/2015 9:10:57 PM
Subject: FW: Glyphosate in honey
survey-of-glyphosate-residues-in-honey-corn-and-soy-products-2161-0525.1000249.pdf

Hi Chris,

(b) (5)

Thanks,

David



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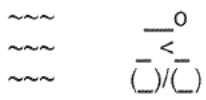

From: Sack, Chris A [mailto:Chris.Sack@fda.hhs.gov]
Sent: Thursday, November 12, 2015 9:25 AM
To: Hrdy, David <Hrdy.David@epa.gov>
Subject: Glyphosate in honey

Hi David,

Requires Consultation with FDA

Thanks

Chris Sack

Residue Expert

Office of Food Safety

Center for Food Safety and Applied Nutrition

US Food and Drug Administration

Phone: 240-402-2464

Environmental & Analytical Toxicology

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Research Article Open Access

Survey of Glyphosate Residues in Honey, Corn and Soy Products

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Abstract

Samples of honey (sixty nine), pancake and corn syrup (twenty six), soy sauce (twenty eight), soy milk (eleven), and tofu (twenty) purchased in the Philadelphia, US metropolitan area were analyzed for glyphosate residue using ELISA. The limit of quantification (LOQ) and range of the method were determined for honey, pancake syrup, and corn syrup to be 15 to 800 ppb; soy sauce, soy milk, and tofu 75 to 4,000 ppb. Glyphosate residues above the limit of quantification were not found in pancake and corn syrup, soy milk, and tofu. Of the sixty-nine honey samples analyzed, forty-one samples, or fifty-nine percent (59%), had glyphosate concentrations above the method LOQ (15 ppb), with a concentration range between 17 and 163 ppb and a mean of 64 ppb. Eleven of the tested honey samples were organic; five of the organic honey samples, or forty-five percent (45%), contained glyphosate concentrations above the method LOQ, with a range of 26 to 93 ppb and a mean of 50 ppb. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62%), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb. In addition to comparison of production method (organic vs. conventional), the honey results were evaluated according to pollen source and by country of origin, grouped by GMO usage (prohibited, limited, or permitted). Glyphosate concentrations above the method LOQ (75 ppb) were also found in ten of the twenty-eight soy sauce samples evaluated (36%), with a concentration range between 88 and 564 ppb and a mean of 242 ppb; all organic soy sauce samples tested were below the method LOQ.

Keywords: Glyphosate; Honey; Honey bees; Soy sauce; ELISA

Introduction

Food consumption is an important pathway of human exposure to pesticides and other chemical contaminants. Studies have shown that exposure to contaminants in food could pose a public health risk [1,2,3]. Contaminants can enter the food supply in various ways including direct pesticide application to food crops, indirect application through the air (from drift from aerial spraying of adjacent fields), through the soil (from direct application during previous growing seasons), through the water supply (from run-off from treated areas), or through food processing (from cross-contamination from shared processing equipment) [4,5].

Glyphosate (N-(phosphonomethyl) glycine) (Figure 1), commonly sold under Monsanto's trade name Roundup®, is a non-selective herbicide that inhibits 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) in the shikimic acid pathway present in plants, bacteria, and archae [6]. EPSP synthase is the rate limiting step in the synthesis of various aromatic acids; inhibition of this enzyme results in depletion of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan [7]. Glyphosate trans locates readily in plants, making it effective for controlling perennial weeds and overwintering rhizomes and tubers. It is registered for pre planting or post harvest treatment on crops and on non-crop land [8]. Although humans do not posses the shikimic acid pathway, we are dependent upon ingested food and gut microbes, which provide essential nutrients, which do possess this pathway. Glyphosate has been patented as an antimicrobial by Monsanto Technology LLC [9], and has been shown to disrupt gut bacteria in animals [10-12]. In humans, only a small amount (~2%) of ingested glyphosate is metabolized to amino methyl phosphonic acid (AMPA), the rest enters the blood stream and is eventually eliminated through the urine [13].

The use of glyphosate in agriculture has increased significantly with the introduction of transgenic crops such as Roundup-Ready® soybeans and corn, which enable farmers to directly apply low cost broad spectrum herbicide products to their fields without harming crops. In the United States, glyphosate is currently the most widely used herbicide, with 180 to 185 millions pounds applied in the agricultural sector during 2007, 5 to 8 million pounds used in homes and gardens, and 13-15 million pounds used in industrial, commercial and governmental weed control applications [13]. The dramatic increase in the use of glyphosate in agriculture and landscape maintenance is occurring not only in the US, but throughout the world. This high level of use has led to concerns about its effects on humans and the environment. Glyphosate has traditionally been considered to be nearly non-toxic to humans [14], and therefore not problematic if ingested in food sources; as a consequence, measurement of its presence in food is very scarce [15,16]. Challenge the assertion that glyphosate is harmless, arguing that this herbicide may be a key contributor to the obesity and autism epidemics in the United States, as well as a factor in several diseases and conditions including celiac disease, Alzheimer's, Parkinson's, infertility, depression, and cancer.

Glyphosate analysis in environmental and biological matrices is problematic because of its small molecular size and structural similarity to many naturally occurring plant materials such as amino acids and secondary plant compounds. It is highly soluble in water, thereby making its extraction with solvents difficult and matrix effects highly prevalent. As a result, glyphosate isolation and quantification has posed a challenge to the analytical chemist. Numerous analytical procedures have been published in the literature for the detection of this highly

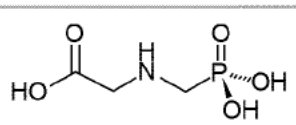

Figure 1: Glyphosate (N-(phosphonomethyl) glycine)

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polar and amphoteric molecule [17], including gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), often coupled with mass spectrometry (MS). The co-contaminants in environmental and biological matrixes render instrumental analysis costly and time-consuming. ELISA determination, however, has allowed for the rapid, selective and sensitive determination of glyphosate [18-24].

The main objective of this survey was to obtain representative data on levels of glyphosate residues in honey, corn and pancake syrup, and soy based products, such as soy sauce, soy milk, and tofu, in products consumed by the general population in and around Philadelphia, Pennsylvania. The analytical program included the extraction of glyphosate from the various matrices and the subsequent determination of glyphosate residues by enzyme linked immune sorbent assay (ELISA).

Materials and Methods

Chemicals and reagents

Chemicals were of reagent grade and were purchased from Sigma Chemical Company, St. Louis MO, USA, except as indicated. Glyphosate (>98% purity), Chem Service, West Chester, PA, USA. Glyphosate micro titer plate ELISA, Abraxis PN 500086; Glyphosate sample diluent, PN 500082, Abraxis LLC, Warminster, PA, USA. Glyphosate stock solution was prepared in deionized water to 1.0 mg/mL; spiking solutions were prepared from the working solution using deionized water.

Samples and sample preparation/extraction

In total, 153 representative samples were purchased from markets in the Philadelphia metropolitan area (69 honey, 26 corn and pancake syrup, 28 soy sauce, 11 soy milk, and 20 tofu products).

Honey, corn and pancake syrup samples: A 0.50 g aliquot of sample was weighed into a micro centrifuge tube and 0.50 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:200 sample dilution.

Soy sauce: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Soy milk: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Tofu: A 1.0 g aliquot of sample was weighed into a 20 mL vial and 10.0 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then allowed to separate for 2 minutes. Approximately 1 mL of the mixture was transferred into a micro centrifuge tube and centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Determination of glyphosate in samples

The instructions provided in the ELISA kit user's guide were followed, in brief, glyphosate calibrators provided in the kit and the samples to be tested are derivatized for ten minutes and then added, along with an antibody specific for glyphosate to micro titer wells coated with goat anti-rabbit antibody and incubated for thirty minutes with shaking. A glyphosate horseradish peroxidase (HRP) enzyme conjugate is then added. At this point a competitive reaction occurs between the glyphosate, in the calibrators or samples, and the enzyme labeled glyphosate for the antibody binding sites on the micro titer well. The reaction is allowed to continue for sixty minutes. After a washing step an enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine) are added. The enzyme-labeled glyphosate bound to the glyphosate antibody catalyzes the conversion of the substrate /chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of diluted acid and read in a Molecular Devices micro titer plate reader (450 nm). Since the labeled glyphosate (conjugate) was in competition with the unlabeled glyphosate (sample) for the antibody sites, the color developed is inversely proportional to the concentration of glyphosate in the sample.

Data analysis

The evaluation of the assay was performed using Molecular Devices Softmax pro evaluation program (4-Parameter). The program calculates the mean absorbance value for each of the standards (B_i) and calculates the $\%B_i/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance (B_0). The program then constructs a non-linear regression model of a standard curve by plotting the $\%B_i/B_0$ for each standard on the vertical linear (y) axis versus the corresponding glyphosate concentration on the horizontal logarithmic (x) axis. The $\%B_i/B_0$ for samples is interpolated using the standard curve yielding sample concentration levels of glyphosate from the standard curve. Correlation coefficients of the assays were >0.995 and standard deviation between standard replicate analysis were < 10%.

Validation, performance and quality control

Specificity had been previously determined (ELISA user's guide), (Table 1). Recovery, limit of quantitation, range and limit of quantification were determined to test the validity of the dilution/extraction procedures of each of the matrices used in combination with the glyphosate ELISA.

COMPOUND (B/Bo)	LOD (ng/mL)	50% B/Bo (ng/mL)
Glyphosate	0.05	0.5
Glyphosine	50	3,000
Glufosinate	2,000	70,000
AMPA	35,000	>1,000,000
Glycine	>10,000	>1,000,000

Table 1: Cross-reactivity table. The reactivity of glyphosate to various related compounds expressed as LOD and as the dose required for 50% absorbance inhibition (50% B/Bo).

Results and Discussion

The method performance for glyphosate analysis was determined by conducting recovery tests on each of the matrices. To determine the accuracy of the glyphosate analysis for the sample matrices analyzed in this study, matrix samples that were glyphosate negative and positive (positive samples were not encountered with tofu, soy milk, pancake and corn syrup) were spiked as follows: 15, 40, 100, 200 and 400 ng/mL (honey, pancake and corn syrup); 75, 200, 500, 1,000 and 4,000 ng/mL [soy sauce, soy milk and tofu (ng/g)]. Analysis was performed in duplicate for all unspiked and spiked samples at all levels. Average recovery obtained for glyphosate negative honey samples fortified with glyphosate was 119 %, (SD = 10). Average recovery for glyphosate positive honey (unspiked contained 44 ng/g glyphosate) after fortification was 116 % (SD = 10). Average recovery for negative soy sauce was 94% (SD = 5), and for positive fortified soy sauce (unspiked contained 417 ng/mL) was 86% (SD = 5). The limit of quantification and range of the method were determined for honey, pancake and corn syrup to be 15 to 800 ng/g; soy sauce, soy milk, and tofu 75 to 4,000 ng/mL or ng/g, respectively.

In this study, the first sample matrix analyzed for the presence of glyphosate was honey; 69 samples were analyzed and classified into 18 groups depending on the country of origin listed on the bottles: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. The glyphosate concentrations obtained are shown in (Figure 2). Forty-one out of the sixty-nine honey samples analyzed, or fifty nine percent (59 %), had glyphosate concentrations above the method LOQ (15 ng/g) with a concentration range between 17 and 163 ng/g and a mean of 64 ng/g.

The glyphosate concentration in honey grouped by flower (pollen) source is shown in (Figure 3). The pollen types listed on the bottles were: clover (12 samples), exotic (11 samples), wildflower (11 samples), unknown (35 samples). (Figure 4) depicts the concentration of glyphosate in honey samples grouped by growing method of source pollen: organic (11 samples) and traditional (58 samples); 5 of the 11 organic samples had glyphosate concentrations above the method LOQ with a range of 26 to 93 ng/g and a mean of 50 ng /g. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62%), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb.

(Figure 5) depicts the concentration of glyphosate in honey by country and whether the use of genetically modified organisms (GMO) seeds is prohibited or permitted. The graph also shows where some minimum uses of GMO traits are allowed (Spain, and blend of Vietnam/Ukraine). The glyphosate concentration in honey originating in countries that do not allow or allow limited GMO traits (3 out of 14 samples above the LOQ) ranged from 26 to 41 ng/g with a mean of 31 ng /g. The glyphosate range for those countries that allow GMO (30 out of 43 samples above LOQ) was 21 to 163 ng/g with a mean of 71 ng /g. Samples of unknown origin (8 out of 12 samples above LOQ) ranged from 17 to 95 ng/g with a mean of 50 ng/g.

The second matrix group analyzed for glyphosate was soy sauce. The analysis consisted of 28 samples, (Figure 6). Ten out of 28 samples (36 %) had glyphosate concentrations above the method LOQ (75 ng/

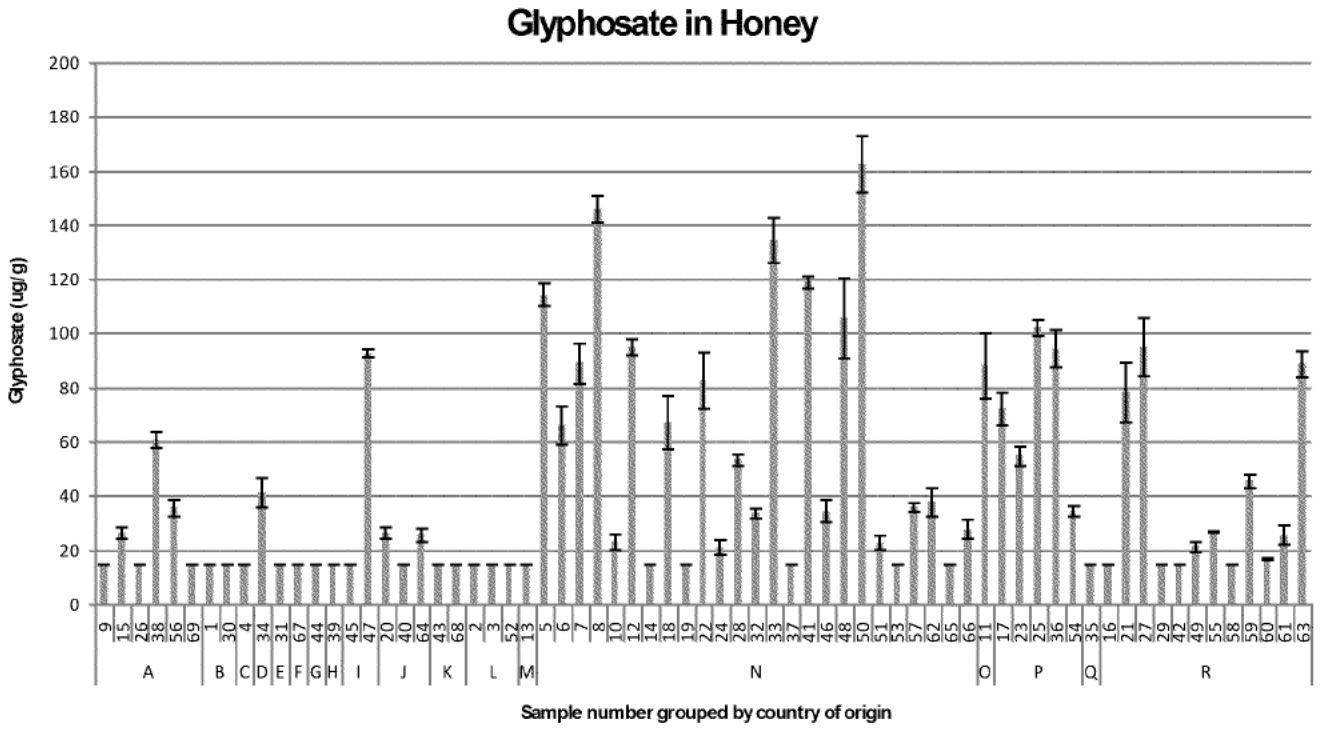

Figure 2: Concentration of glyphosate (ng/g) in honey samples listed by honey origin: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g). Error bars represent concentrations obtained during duplicate analysis.

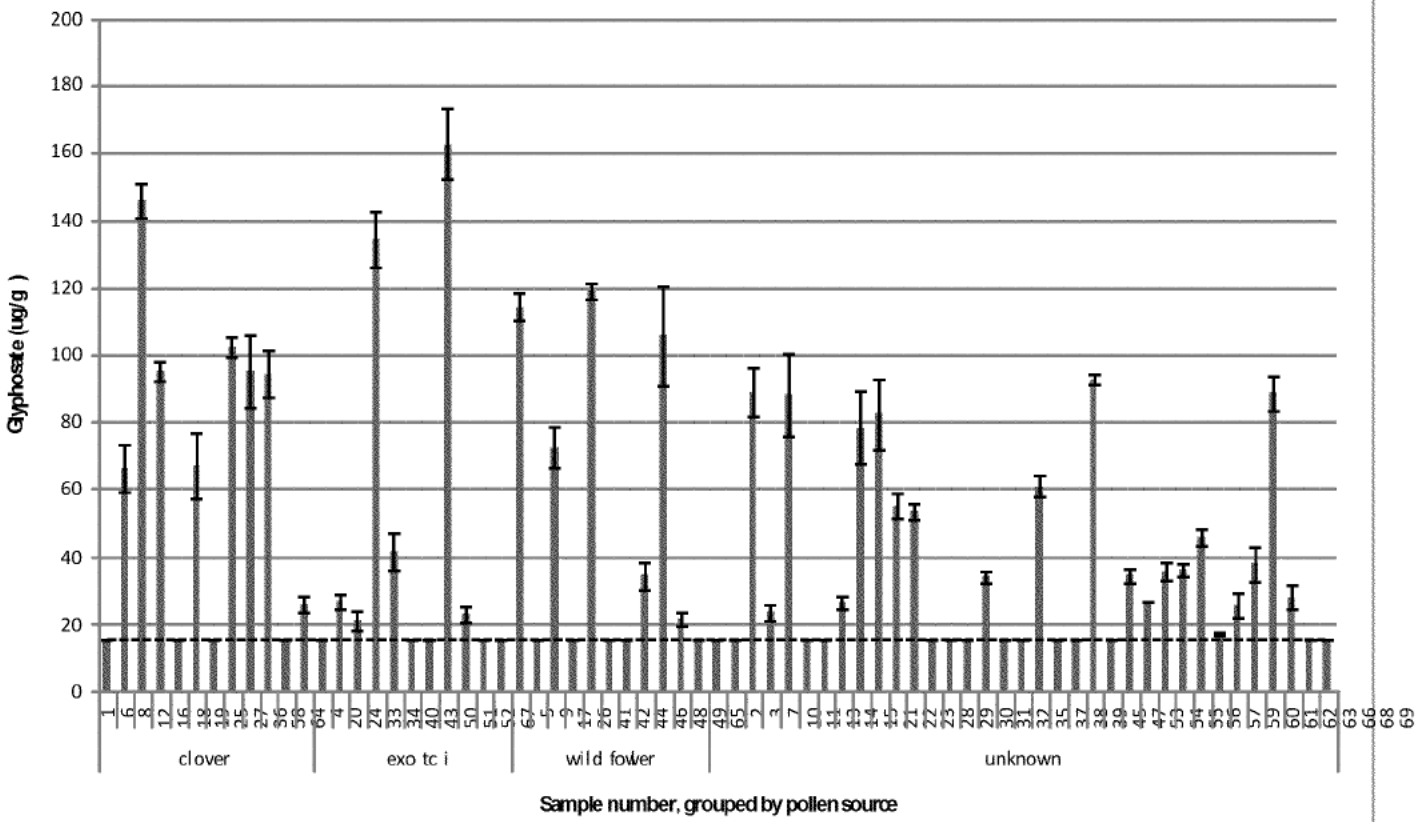

Figure 3: Concentration of glyphosate (ng/g) in honey samples by flower (pollen) source. Dashed line represents LOQ of method (15 ng/g). Exotic flowers were sophora, manuka, orange, cactus, summer flower, lychee, alfalfa, acacia.). Error bars represent concentrations obtained during duplicate analysis.

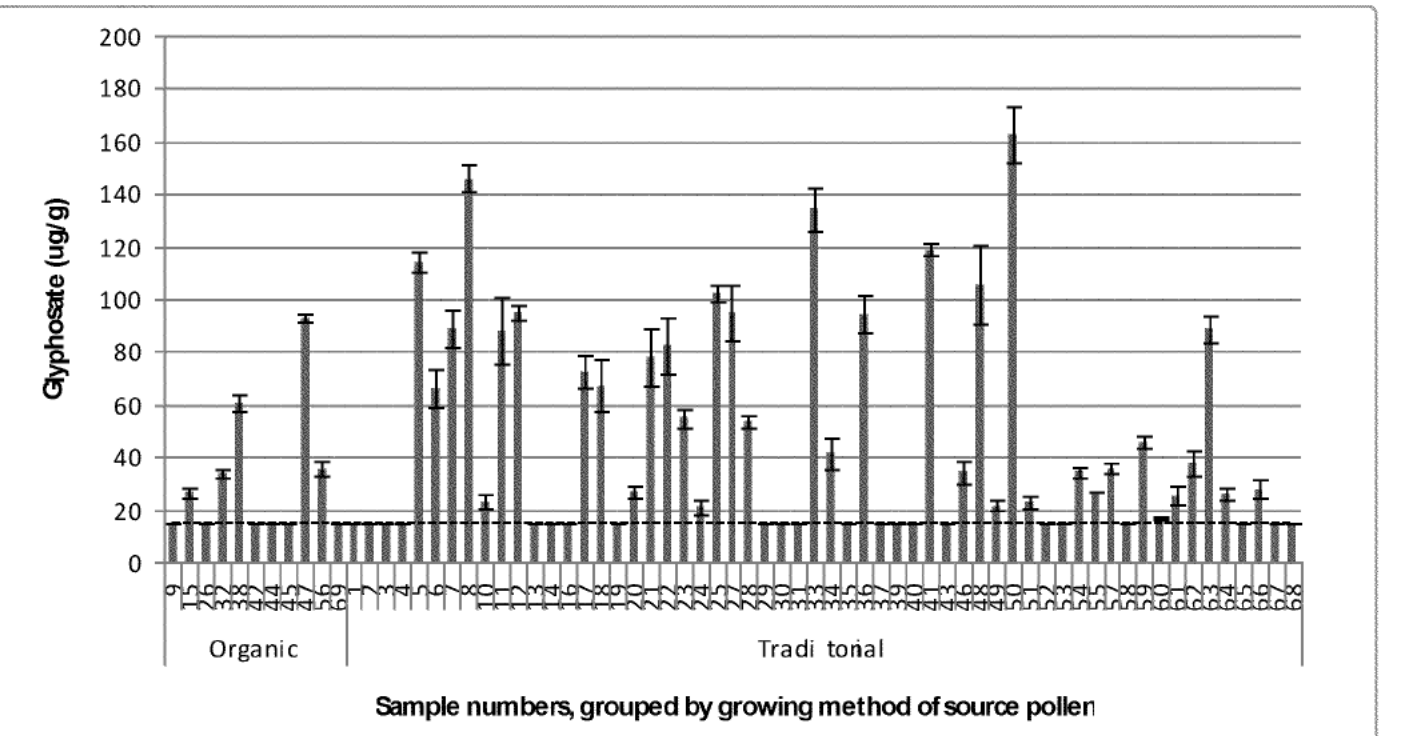

Figure 4: Concentration of glyphosate (ng/g) in honey samples by growing method of source pollen (Organic vs.Traditional). Dashed line represents LOQ of method (15 ng/g).) Error bars represent concentrations obtained during duplicate analysis.

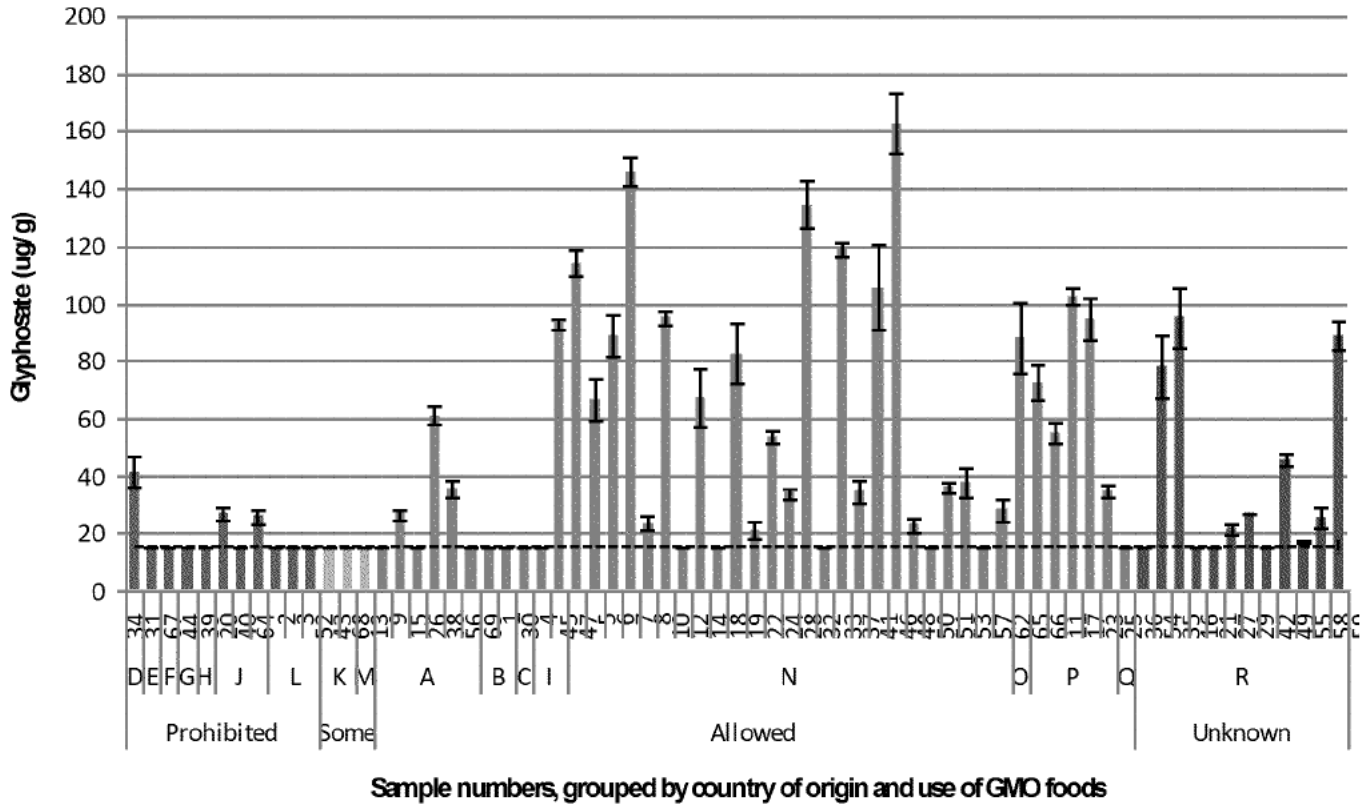

Figure 5: Concentration of glyphosate (ng/g) in honey samples listed by honey origin and the allowance of GMO use: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g).). Error bars represent concentrations obtained during duplicate analysis.

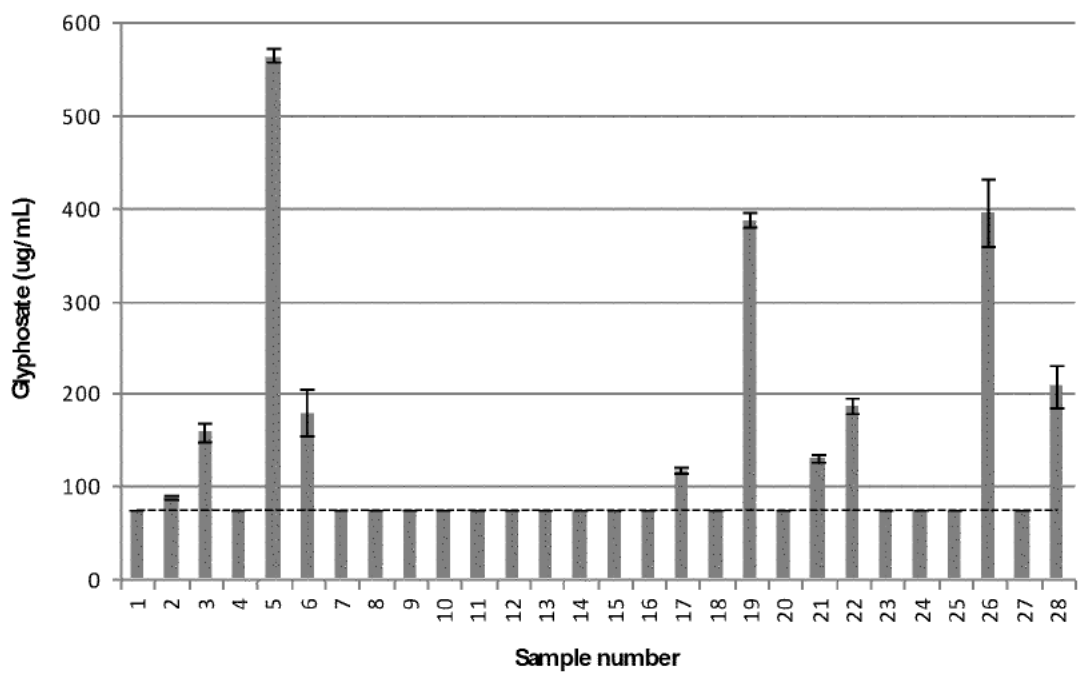

Figure 6: Concentration of glyphosate (ng/mL) in soy sauce samples. Dashed line represents LOQ of method (75 ng/mL).). Error bars represent concentrations obtained during duplicate analysis.

mL) with a concentration range between 88 and 564 ng/mL and a mean of 242 ng /mL. (Figure 7) shows the concentration of glyphosate in soy sauce by method of soy bean growing (organic vs. traditional). Therecent report from the Chinese Academy of Medical Science and the Beijing Union Hospital [20] reported an average glyphosate concentration in soy sauce of 133 ng/mL in samples that did not specify on the bottle whether or not the raw material was GM soybean. In our study, the small subset of organic labeled samples (three) was all below the limit of quantitation of the test.

Corn and pancake syrup (26 samples), soy milk (11 samples), and tofu (20 samples) tested were negative for glyphosate at the LOQ of the method (15 ng/g for pancake and corn syrup, and 75 ng/mL or ng/g for soy milk and tofu, respectively).

Studies on glyphosate residues in food are scarce. Among the few studies found was a recent report published on the incidence of glyphosate in soy sauce, conducted by the Chinese government [20]. Searches were conducted by the authors using various scientific databases on the concentration and incidence of glyphosate in honey, but these failed to provide any information. Thehoney samples analyzed in the present study show that 59 % of all samples contained glyphosate residues (ranging from 17 to 163 ng/g, mean 64 ng/g); the residue concentration does not seem to depend on pollen source or growing method, even organic honey contained glyphosate residues (5 out 11 samples, or 45 %, mean glyphosate concentration 50 ng/g). Comparing the concentration of glyphosate in honey by countries that use GMO extensively with countries that allow the use of some GMO traits and those that do not allow GMO, shows that, in general, glyphosate levels are lower in samples from countries that do not allow or allow limited use of some GMO traits, such as Spain and Vietnam/ Ukraine blend (mean 31 ng/g), compared to those countries that allow planting of GMO traits (71 ng/g). It should be noted, however, that some residues of glyphosate (although < 50 ng/g) were found in honeys originating from Germany and New Zealand, countries where no GMO planting is allowed.

TheEuropean Union has specificguidelines for the labeling of organic honey [25,26]. According to those guidelines, the location of apiaries is strictly controlled and states that "Nectar and pollen sources available over a three-kilometer radius around the apiary sites must consist essentially of organically produced crops or crops treated with low-environmental-impact methods. Apiaries must also be far enough away from any non-agricultural production source that could lead to contamination (e.g. urban centers, waste dumps, waste incinerators, etc.). Member States have the option of prohibiting the production of organic honey in certain regions or areas that do not meet these conditions. Organic honey must not contain chemicals residues (synthetic pesticides, etc.)." TheUnited States has no such guidelines for the organic production of honey, but uses organic farming certification for honey labeling purposes; one reason is that it is practically impossible to regulate without testing all honey for residues since bees can flyup to 3 miles in search of nectar and it is difficult to be certain that they do not feed on nectar contaminated by crop spraying or industrial sources. In the EU, glyphosate residues in non-organic honey regulatory limits are 50 ng/g [27], the United States does not have a limit in honey. Thelimit in drinking water in the United States is 700 ng/mL; the reference dose is 1.75 mg/Kg/day; the One-Day Health Advisory level is 20 mg/L [28]. Also, it is widely known that like milk and olive oil, honey is one of the foods that is most commonly mislabeled and adulterated [29] providing yet another source of glyphosate contamination in honeys that, according to the bottle label, originated in non-GMO countries.

Bee colony collapse disorder (CCD) is a growing threat to the efficient production of food around the world. Honey bees pollinate nearly 130 species of plant life [30], such as fruits, vegetables, nuts, and seed crops. Honeybees are therefore indirectly responsible for an

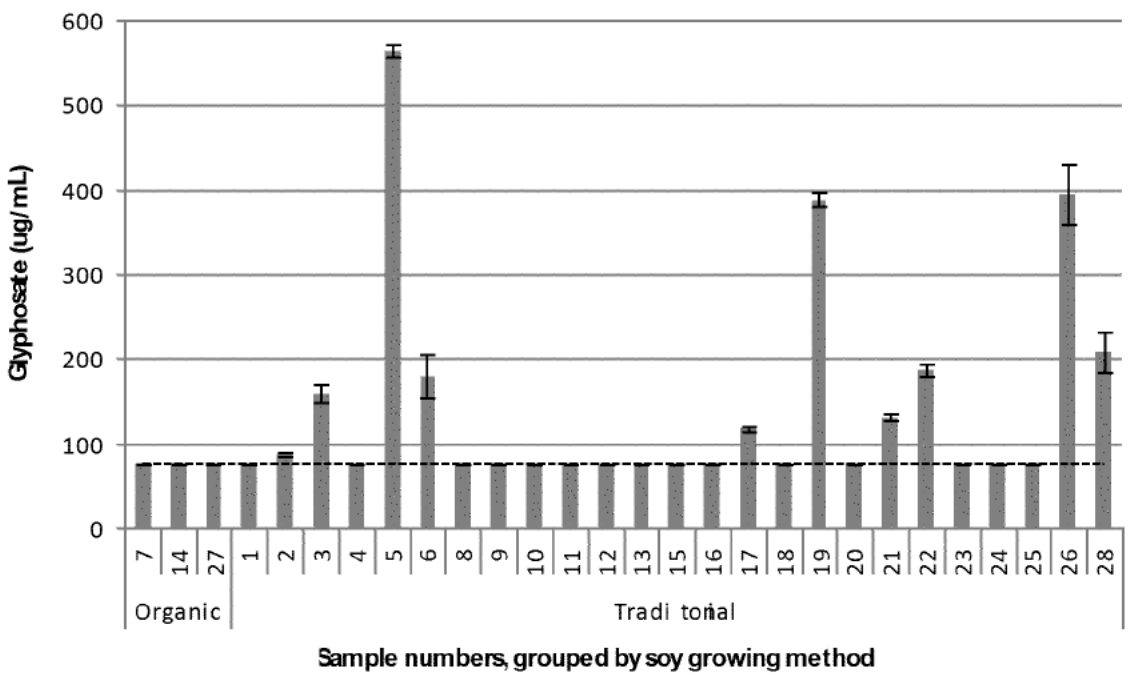

Figure 7: Concentration of glyphosate (ng/mL) in soy sauce samples by growing method of soy beans (Organic vs.Traditional). Dashed line represents LOQ of method (75 ng/mL).). Error bars represent concentrations obtained during duplicate analysis.

estimated one-third of the world food supply [31]. Although several factors are involved in CCD, including numerous pathogens and parasites, the extensive use of pesticides [32,33] such as neonicotinoids have provided evidence that these products are harmful to honey bees and have lead to a recent ban or restriction in the use of three neonicotoids by the European Union [34]. Although glyphosate is not acutely toxic to bees, it is chronically toxic to animals and is reported to disrupt the endocrine system [35,36] and a recent study indicates that honey bees exposed to increasing sub-lethal concentrations of glyphosate exhibit a decrease in acetyl cholinesterase (AChE) activity [37]. Thehigh rate of glyphosate use creates the potential for wide-spread contamination of our food chain. Glyphosate is used throughout the bee foraging period in high amounts and is found in the air, water, and in plant parts frequented by bees, such as flowers and buds, potentially contaminating the nectar collected by bees from contaminated plants [38]. Based on its prevalence in the environment, as well as our findingsin honey samples, we propose that future studies should be conducted to determine if glyphosate is in fact a contributing factor in CCD.

Conclusion

Thistudy indicates the presence of glyphosate residues in honey and soy sauce, but not in pancake and corn syrups or soy based products such as soy milk and tofu. Forty one out of sixty nine (59%) honey samples analyzed contained glyphosate at a concentration above the method LOQ (15 ng/g) with a range between 17-163 ng/g and a mean of 64 ng/g. Ten out of twenty eight (36%) soy sauce samples contained glyphosate at a concentration above the method LOQ (75 ng/mL) with a range between 88-564 ng /mL and a mean of 242 ng /mL. Future studies should be conducted on many other food products to determine the extent of glyphosate residue contamination.

Acknowledgements

We would like to give our thanks to Erin Faltin for the preparation of the graphs presented in this paper.

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Citation: Rubio F, Guo E, Kamp L (2014) Survey of Glyphosate Residues in Honey, Corn and Soy Products. J Environ Anal Toxicol 4: 249. doi: 10.4172/2161-0525.1000249

To: Hrdy, David[Hrdy.David@epa.gov]; Miller, David[Miller.DavidJ@epa.gov]
From: Councell, Terry
Sent: Thur 11/12/2015 1:49:24 PM
Subject: FW: UNDER EMBARGO - Glyphosate: EFSA updates toxicological profile
Glyphosate_Layman_FINAL.pdf
Glyphosate_Complementary information_FINAL.pdf
Glyphosate_Peer_News Story_FINAL.pdf

FYI

From: Dietz, Jason
Sent: Thursday, November 12, 2015 8:48 AM
To: Robin, Lauren P; Councell, Terry
Subject: FW: UNDER EMBARGO - Glyphosate: EFSA updates toxicological profile

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From: LIEM Djien [mailto:Djien.LIEM@efsa.europa.eu] **On Behalf Of** international.cooperation
Sent: Thursday, November 12, 2015 2:56 AM
Subject: UNDER EMBARGO - Glyphosate: EFSA updates toxicological profile

Dear Colleagues,

Today 12 November at 12:00 CET, EFSA will publish a Conclusion on the Peer review on glyphosate and a complementary technical document.

It will be accompanied by a News Story and a non technical summary.

The documents are under embargo until **12:00 CET** when they will be published on our website.

For any further information on the Conclusion, please contact Jose Tarazona (Jose.Tarazona@efsa.europa.eu).

For any further information on the News Story, please contact Simon Terry (simon.terry@efsa.europa.eu).

Best regards,

Djien

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intended to be shared beyond recipients identified in the distribution list above until the final documents are actually published. There is always a possibility that there will be additional changes before the final version is published and that the actual date and/or time of publication, indicated by the embargo, may change. Please note that only the final, published version remains the reference document. The EFSA website should be checked for confirmation of final content and publication. Only documents which are published on EFSA's website can be cited/used.

UNDER EMBARGO until 12/11/2015 at 12.00 CET
NOT FOR FURTHER DISTRIBUTION

Parma, 12 November 2015

NEWS STORY

Glyphosate: EFSA updates toxicological profile

EFSA and the EU Member States have finalised the re-assessment of glyphosate, a chemical that is used widely in pesticides. The report concludes that glyphosate is unlikely to pose a carcinogenic hazard to humans and proposes a new safety measure that will tighten the control of glyphosate residues in food. The conclusion will be used by the European Commission in deciding whether or not to keep glyphosate on the EU list of approved active substances, and by EU Member States to re-assess the safety of pesticide products containing glyphosate that are used in their territories.

A peer review expert group made up of EFSA scientists and representatives from risk assessment bodies in EU Member States has set an acute reference dose (ARfD) for glyphosate of 0.5 mg per kg of body weight, the first time such an exposure threshold has been applied to the substance.

Jose Tarazona, head of EFSA's Pesticides Unit, said: "This has been an exhaustive process – a full assessment that has taken into account a wealth of new studies and data. By introducing an acute reference dose we are further tightening the way potential risks from glyphosate will be assessed in the future. Regarding carcinogenicity, it is unlikely that this substance is carcinogenic."

Unlikely to be carcinogenic

The peer review group concluded that glyphosate is unlikely to be genotoxic (i.e. damaging to DNA) or to pose a carcinogenic threat to humans. Glyphosate is not proposed to be classified as carcinogenic under the EU regulation for classification, labelling and packaging of chemical substances. In particular, all the Member State experts but one agreed that neither the epidemiological data (i.e. on humans) nor the evidence from animal studies demonstrated causality between exposure to glyphosate and the development of cancer in humans.

EFSA also considered, at the request of the European Commission, the report published by the International Agency for Research on Cancer (IARC), which classified glyphosate as probably carcinogenic to humans.

The evaluation considered a large body of evidence, including a number of studies not assessed by the IARC which is one of the reasons for reaching different conclusions.

As well as introducing the ARfD, the review proposed other toxicological safety thresholds to guide risk assessors: the acceptable operator exposure level (AOEL) was set at 0.1 mg/kg bw per day and an acceptable daily intake (ADI) for consumers was set in line with the ARfD at 0.5 mg/kg bw.

Next steps

The EFSA conclusion will inform the European Commission in deciding whether or not to retain the substance on the EU's list of approved active substances. This is a condition for enabling Member States to authorise its continued use in pesticides in the EU.

For an explanation of the main findings of EFSA's Conclusion on glyphosate, please consult our special non-specialist summary. EFSA has also produced a supplementary document that takes an in-depth look at some of the scientific issues that were highlighted during the assessment.

Background

Glyphosate is an active chemical substance widely used in a number of pesticide products and its use in Europe is subject to strict regulation. The EFSA peer review was carried out as part of the legal process required to renew authorisation of its use in Europe.

Following standard procedure for such renewals, the applicants supplied a dossier of relevant scientific information to a Member State – known as the rapporteur Member State (RMS) – to carry out an initial assessment. The dossier was assessed by the RMS, in this case Germany, and passed to EFSA to make a final evaluation of the evidence and conclusions in the file. This process is carried out by a peer review group made up of EFSA scientists and representatives from risk assessment bodies in all EU Member States.

What are maximum residue levels?

A maximum residue level (MRL) is the highest concentration of an active substance that is legally tolerated in food or feed when pesticides are applied correctly. EFSA is responsible for proposing MRLs in the EU, assessing the safety for consumers based on the toxicity of the pesticide, the levels expected to be found in food and the different food consumption habits of Europeans. The safety assessment is carried out using toxicological reference values such as the acute reference dose (ARfD).

What is an acute reference dose (ARfD)?

An ARfD is an estimated intake of a chemical substance in food, expressed on a bodyweight basis, that can be ingested over a short period of time, usually during one meal or one day, without posing a health risk.

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EFSA explains risk assessment

Glyphosate

Glyphosate is an active substance that is widely used in pesticides. Glyphosate-based pesticides (i.e. formulations containing glyphosate and other chemicals) are used in agriculture and horticulture primarily to combat weeds that compete with cultivated crops. They are typically applied before crops are sown and as a pre-harvest desiccating treatment, accelerating and evening the ripening process.

What has EFSA done?

EFSA and EU Member States have carried out a risk assessment and peer review that updates our scientific knowledge of the toxicity of glyphosate. EFSA has published a Conclusion on glyphosate as part of this process. The conclusion will be used by the European Commission in deciding whether or not to keep glyphosate on the EU list of approved active substances, and to inform the subsequent evaluations by Member States on the use of glyphosate-based formulations in their territories. (See box: "How is the safety of glyphosate assessed in the EU?")

Main findings of the assessment

After considering the huge amount of relevant data, an EFSA peer review expert group made up of EFSA scientists and representatives nominated by EU Member States concluded that:

- The toxicity of glyphosate needs to be redefined. An acute reference dose (ARfD) of 0.5 mg/kg of body weight has therefore been proposed, the first time such a safety measure has been introduced for glyphosate. EFSA will use this ARfD during its review of the maximum residue levels for glyphosate, which will be carried out in cooperation with Member States in 2016. The acceptable operator exposure level (AOEL) has also been set at 0.1 mg/kg body weight per day and an acceptable daily intake (ADI) for consumers has been set in line with the ARfD at 0.5 mg/kg body weight per day.
- The substance is unlikely to be genotoxic (i.e. damaging to DNA) or to pose a carcinogenic threat to humans. Glyphosate is not proposed to be classified as carcinogenic under the EU regulation for classification, labelling and packaging of chemical substances. In particular, all the Member State experts but one agreed that neither the epidemiological data (i.e. on humans) nor the evidence from animal studies demonstrated causality between exposure to glyphosate and the development of cancer in humans.

How is the safety of pesticides assessed in the EU?

Under EU legislation, pesticide active substances in plant protection products are approved in the EU only if it may be expected that their use will not have any harmful effects on human and animal health or the environment.

The evaluation of both existing and new active substances follows a phased approach:

1. For each substance an initial draft assessment report (DAR) or renewal assessment report (RAR) is produced by a designated rapporteur Member State (RMS). Regarding applications for renewal of an approval, the Commission decides on the designation of a rapporteur Member State in consultation with all Member States and industry.
2. The RMS's risk assessment is peer reviewed by EFSA in cooperation with all Member States.
3. EFSA drafts a report ("Conclusion") on the active substance. The EFSA Conclusion informs the European Commission in the approval process, the subsequent assessments of plant protection products by the Member States, and the revision of maximum residue levels in food by EFSA.
4. The European Commission decides whether or not to include the substance in the EU's list of approved active substances. This determines whether the substance can be used in a plant protection product in the EU.
5. EU Member States assess or re-assess the safety of pesticides containing the active substance that are sold in their territory.

Why do some scientists say that glyphosate is carcinogenic?

The International Agency for Research on Cancer (IARC) said earlier this year that glyphosate was genotoxic and would "probably" cause cancer in humans.

However, the IARC report looked at both glyphosate – an active substance – and glyphosate-based formulations, grouping all formulations regardless of their composition. The EU assessment, on the other hand, considered only glyphosate. Member States are responsible for evaluating each plant protection product that is marketed in their territories.

This is because the EU and IARC take different approaches to the classification of chemicals. The EU scheme –assesses each individual chemical, and each marketed mixture separately. IARC assesses generic agents, including groups of related chemicals, as well as occupational or environmental exposure, and cultural or behavioural practices.

This is important because although some studies suggest that certain glyphosate-based formulations may be genotoxic (i.e. damaging to DNA), others that look solely at the active substance glyphosate do not show this effect. It is likely, therefore, that the genotoxic effects observed in some glyphosate-based formulations are related to the other constituents or "co-formulants". Similarly, certain glyphosate-based formulations display higher toxicity than that of the active ingredient, presumably because of the presence of co-formulants. In its assessment, EFSA proposes that the toxicity of each

pesticide formulation and in particularits genotoxic potential should be further considered and addressed by Member State authorities while they re-assess uses of glyphosate-based formulations in their own territories.

This distinction between active substance and pesticide formulation mainly explains the differences in how EFSA and IARC weighed the available data. For the EU assessment, studies conducted with glyphosate were more relevant than studies conducted with formulated products containing other constituents, particularly when the other constituents could not be clearly identified.

What data was used in this assessment?

The EFSA-led review considered a large body of evidence, including the IARC report. In addition to the original studies submitted by the applicants in line with the legal requirements, all available and published studies were considered.

IARC included a number of epidemiological studies in its monograph that were absent from the draft EU assessment; these studies were later added to the EU dossier.

In total EFSA assessed more evidence including additional key studies that were not considered by IARC.

How were the animal studies on carcinogenicity interpreted?

The EU peer review concluded that no significant increase in tumour incidence could be observed in any of the treated groups of animals in the nine long term rat studies considered. IARC, on the other hand, interpreted two studies as showing statistically significant carcinogenic effects. Similarly, with the mice studies, IARC identified positive carcinogenic trends in two studies that the EU peer reviewers assessed as insignificant.

The main differences between the EFSA and IARC evaluations are explained in detail in a special background document published by EFSA. As well as reviewing a larger number of studies, EFSA for example considered that carcinogenic effects observed at high doses were unreliable as they could be related to general toxicity.

What happens next?

The EFSA conclusion will inform the European Commission in deciding whether or not to retain the active substance glyphosate on the EU's list of approved active substances, in other words to authorise its continued use in pesticides in the EU.

Timeline

2012

May — Germany, as rapporteur member state (RMS), receives dossier in support of the possible renewal of the authorisation of glyphosate.

2013

December	RMS sends draft renewal assessment report (RAR) to EFSA.
2014	
January	Peer review begins. RAR sent to Member States and applicants for consultation and comments.
March	Public consultation launched, lasting 60 days.
July	RMS evaluates all comments.
August	Additional information requested from applicants.
2015	
Feb–March	EFSA organises expert consultations in the areas of mammalian toxicology, residues, environmental fate, and ecotoxicology
July	Member State consultation is launched on conclusions arising from peer review.
	IARC monograph published.
August	RMS prepares assessment of the monograph, which is circulated to Member States for comments.
September	Following receipt of comments, EFSA organises second expert consultation on carcinogenicity and mammalian toxicology.
October	Glyphosate authorisation provisionally extended until June 2016, pending finalisation of EU peer review
October	Peer review updated accordingly and final consultation takes place with Member States.

What do we mean by...

Active substance

An active substance is any chemical, plant extract, pheromone or micro -organism that acts against "pests" on plants, parts of plants or plant products.

Acute reference dose (ARfD)

An ARfD is an estimate of a chemical substance in food, expressed on a bodyweight basis, that can be ingested over a short period of time, usually during one meal or one day, without posing a health risk.

Co-formulant

Pesticides are marketed in different formulations that consist of the active substance and various co-formulants. Glyphosate can be used in combination with POE-tallowamine, a co-formulant that promotes the penetration of the active substance into plants.

Genotoxicity

Genotoxic chemicals damage the genetic information within a cell (DNA), causing mutations that may lead to cancer.

Maximum residue levels

A maximum residue level (MRL) is the highest concentration of an active substance that is legally permitted in food or feed when pesticides are applied correctly. EFSA is responsible for proposing MRLs in the EU.

Metabolite

Metabolites are breakdown products that form when a pesticide mixes with air, water, soil or living organisms. They are considered in EFSA's pesticide safety assessments

Weight of evidence

When there are many studies available on a subject, it is good practice to integrate all the available information and identify consistencies and inconsistencies in the results, then weigh the results according to their reliability and relevance.

EFSA explains the carcinogenicity assessment of glyphosate

12 November 2015

Background

During the EFSA peer-review process for the renewal of the approval of the pesticide active substance glyphosate, EFSA received a complementary mandate from the European Commission to consider the findings by the International Agency for Research on Cancer (IARC) regarding the potential carcinogenicity of glyphosate or glyphosate containing plant protection products. Following the request, EFSA has incorporated its scientific assessment in the on-going peer review of the active substance (EFSA, 2015a).

The EFSA Conclusions on Pesticides have a complex structure, designed for supporting the European Commission and Member States in the approval process, the subsequent assessments of the Plant Protection Products (PPPs) by the Member States (MSs), and the revision of the Maximum Residue Levels (MRLs) of pesticides.

The EFSA Conclusions summarise the main outputs of the scientific assessments (Sections 1 to 6), and then focus on the identification of data gaps and studies to be generated (Section 7), recommendations to manage the identified risks (Section 8) and concerns to be considered by risk managers (Section 9). An appendix presents the List of Endpoints recommended by EFSA for the hazard and the risk assessment of the active substance.EFSA also publish supporting background documents providing the scientific justifications.

EFSA's assessment on pesticide active substances is based on original studies (mandatory regulatory Good Laboratory Practice (GLP) studies, other relevant studies and the outcome of the search of peer-reviewed scientific studies published within the last 10 years before the submission of the dossier), that are summarised by the rapporteur Member State (RMS) in the draft renewal assessment report (RAR). The peer-review includes a public consultation of the draft RAR and several commenting phases by EFSA scientists and MSs experts, the possibility for requiring additional information from the applicants, and a set of experts' meetings covering different scientific areas; this is reflected in a further development of the RMS RAR during the EFSA peer review.

Due to the length and complexity of the peer review report supporting the EFSA Conclusion (EFSA, 2015b) and the RAR (Germany, 2015) EFSA has considered important to clarify some key elements of its scientific evaluation in this complementary document, in particular, regarding the assessment of the carcinogenicity of the active substance glyphosate.

Assessment of the carcinogenic potential of the active substance glyphosate

EFSA has assessed the carcinogenicity and genotoxicity potential of the active substance glyphosate according to the principles and criteria applicable for the classification and labelling of chemical substances in the EU under Regulation (EC) No 1272/2008 (CLP

Regulation)[1]. This regulation implements in the EU the Globally Harmonized System developed by the United Nations for the classification and labelling of hazardous chemical substances. The European Chemicals Agency (ECHA) has developed guidance on the application of these criteria (available in the ECHA web page) and submitted specific comments regarding the Rapporteur Member State (RMS) assessment (EFSA, 2015b) that were considered by EFSA and the MSs during the peer review. Glyphosate carcinogenicity was discussed in two experts' meetings, first during the overall assessment of the mammalian toxicity and second during a dedicated experts' teleconference, involving a large number of experts.

Genotoxicity

The genotoxicity data package available for the peer review is comprehensive. A large number of studies provided in the draft RAR (over 100 studies all together; Germany, 2013) was complemented by additional studies identified during the commenting period and public consultation that took place in 2014, and which were included in the revised RAR (Germany, 2015).

a) glyphosate

EFSA assessed the genotoxic potential of the active substance, glyphosate, and focused on the studies performed on well characterized test substances as manufactured. Glyphosate is produced by many different companies, each manufacturing a technical material presenting different impurity profiles than the others. Where considered necessary, data gaps have been set for each individual company to complete the toxicological information on the impurities, which are present in their own technical material (EFSA, 2015a).

All required genotoxicity endpoints that consist of gene mutation in bacterial and mammalian cells, structural and numerical chromosome aberrations *in vitro* and *in vivo* have been investigated in validated OECD guideline-compliant studies following the principles of GLP, as well as published studies often following non-GLP protocols designed by the study authors.

In vitro tests

Bacterial and mammalian cells mutagenicity studies gave consistently negative results; even studies that were considered less reliable and of lower quality did not reveal any indication of genotoxicity. With regards to *in vitro* mammalian chromosome aberration (CA), all tests that were performed under GLP conditions, as well as a number of published studies gave negative results at concentrations up to 1250 µg/L. In contrast positive results were obtained in some published studies on CA at lower dose levels, and on other endpoints considered as indicator tests, such as sister chromatid exchange (SCE) and induction of DNA strand breaks *in vitro*. The positive indications observed in some *in vitro* tests were not confirmed by *in vivo* studies covering the appropriate end-points, such as *in vivo* micronucleus tests.

In vivo tests

Sixteen *in vivo* studies in somatic cells were reported on rodents treated orally with dose levels up to 5000 mg/kg bw or via intraperitoneal injections. All studies conducted according to internationally validated guidelines and some non-GLP published studies gave negative results, while two non-GLP studies resulted positive in mice treated intraperitoneally with dose levels in the range of the intraperitoneal LD_{50} for mice, one study presenting major flaws. Conflicting results were obtained regarding DNA adduct

[1] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008 p.1-1355

formation; induction of DNA strand breaks was observed in mice treated intraperitoneally with doses close to or in excess of the LD_{50}, this induction may be caused by secondary effects of cytotoxicity. No genotoxic effects on germ cells have been detected in rats or mice treated orally at dose levels up to 2000 mg/kg bw.

Considering a weight of evidence approach, taking into account the quality and reliability of all available data, it is concluded that glyphosate is unlikely to be genotoxic *in vivo* and does not require hazard classification regarding mutagenicity according to the CLP Regulation. It is noted that unpublished studies that were the core basis of the peer review evaluation were not available to the IARC experts as reported in the IARC monograph 112 on glyphosate (IARC, 2015).

b) glyphosate-based formulations

A number of published studies performed with glyphosate based formulations of unknown composition gave positive results when tested *in vitro* and *in vivo*. Some of the test systems are not validated and/or of difficult interpretation due to possible confounding, such as cytotoxicity, specific organ toxicity or unclear relevance to human health when tested in fish, amphibians or invertebrates according to the current knowledge. POE-tallowamine is one of the co-formulants that is known to be used in some glyphosate-based formulations. This co-formulant has been shown to be more toxic than the active substance glyphosate on several toxicological endpoints, namely acute, short term, reproductive and developmental toxicity, further to equivocal evidence of DNA damage *in vitro* at high doses (EFSA, 2015c). Although POE tallowamine is not present in the representative formulation, for which data have been submitted under the European re-approval procedure and which were assessed by EFSA, the peer review concluded that the toxicity of formulations and in particular their genotoxic potential should be further considered and addressed.

It is noted that a number of human studies were not evaluated, since the exposure was linked to glyphosate-based formulations of unknown composition. Therefore their assessment would not have changed the overall conclusion.

Carcinogenicity

a) Studies in animals

Long term toxicity and carcinogenicity potential of glyphosate was assessed in five mouse studies and nine rat studies; one further study in mice and one in rats were considered inadequate for the evaluation of glyphosate carcinogenicity.

In the nine long term rat studies, the EU peer review concluded that no significant increase in tumour incidence was observed in any of the treated groups of animals. Three of these studies were not evaluated by the IARC experts. In one study, the IARC reported a statistically significant increased incidence of pancreatic islet cell adenomas in males treated with the low dose, which was not reproduced at higher dose levels. In another study, the IARC reported significant increases in pancreatic islet adenomas in males at two dose levels (not dose-related), a significant positive trend for hepatocellular adenomas in males without progression to malignancy and a significant trend for C-cell adenomas in females. The two evaluations differ primarily regarding the statistical evaluation: according to a pair-wise comparison [Fisher´s exact test (one-tailed) as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05) and Peto Analysis for evaluation of histopathological data as planned in the study protocol] no significant change is observed, while a trend analysis (Cochran-Armitage trend test) performed by the IARC experts identified significant changes. EFSA is of the opinion that the planning of a study before the initiation of the experimentation itself as established in the respective protocol –that includes the statistical analysis - is a key element in assessing

the quality of a study, therefore deviations from the statistical analysis used by the study authors should be limited and properly justified. Furthermore, clear dose-response was not always observed and when observed, it affected only the highest dose level of 940 to 1183 mg/kg bw per day in males and females respectively, a dose eliciting other adverse effects on body weight, liver, stomach mucosa and eyes (cataracts).

In mice, the EU peer review evaluated five studies; another study was considered inadequate for the evaluation of glyphosate carcinogenicity. From these studies, only one study in Swiss albino mice presented a carcinogenic effect characterised by a statistically significant increased incidence of malignant lymphomas at the top dose level of 1460 mg/kg bw per day. However, the validity of the study was questioned due to the occurrence of viral infection that could influence survival as well as tumour incidence – especially lymphomas. No other carcinogenic effects were observed up to the highest dose levels of each of the other studies as tumour incidences remained within valid historical control data from the respective performing laboratory and/or did not attain the level of statistical significance. The IARC evaluated two of these studies and identified positive trend in males for renal tubule adenomas and carcinomas in one study and positive trend for haemangiosarcoma in the other study according to Cochran-Armitage trend test. EFSA adopted a weight of evidence approach which was agreed by the peer review taking into account all available data: the statistical significance found in trend analysis (but not in pair-wise comparison) was balanced against the lack of consistency in multiple animal studies, slightly increased incidences only at dose levels at or above the limit dose of 1000 mg/kg bw per day recommended for the oral route of exposure in chronic toxicity and carcinogenicity studies (OECD, 2012a), doses at which confounding of concomitant toxicity is expected, incidences within valid historical control range from the performing laboratory and lack of pre-neoplastic lesions.

Furthermore no genotoxic potential is attributed to the active substance glyphosate. Other mechanisms of action were reported by IARC, such as inflammation, immunosuppression, endocrine disrupting (ED) activity and oxidative stress. Although no robust information is available to conclude on the immunomodulatory potential of glyphosate, indications of such effects were limited to inflammatory responses of the respiratory tract. No interaction of glyphosate with the oestrogen, androgen or thyroid endocrine pathways were identified by the US EPA Endocrine Disruptor Screening Program (EDSP) according to a full battery of Tier I screening assays (data gaps were however identified in the EFSA conclusion for the submission of the respective studies for confirmation); and no significant ED effects were identified in published studies reported by IARC, except for a higher activity of formulations in comparison to the active substance. Some indications of increased oxidative stress were observed in combination with cytotoxic or degenerative effects of the target organs; however, even if these indications are confirmed, the observation of a plausible mechanism of action *per se* is insufficient to assume a carcinogenic potential in humans according to the current knowledge. On this basis, EFSA concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans and no classification regarding carcinogenicity is proposed according to CLP Regulation.

b) Epidemiological studies

Overall thirty epidemiological studies are reported in the IARC monograph, including cohort, case-control studies and meta-analyses. A few of these studies were not reported in the RAR (Germany, 2013), but were considered by the RMS in an addendum on the assessment of the IARC monograph (Germany, 2015). In ten cohort studies, that include the Agricultural Health Study (AHS) (Alavanja *et al.*, 1996), the largest prospective cohort study undertaken until today and used in many other publications, glyphosate did not cause different types of cancer and did not increase the risk of all cancers. Nine case-control studies did not indicate an increased risk of carcinogenicity by glyphosate, or presented limited power. Further five case-control and one prospective cohort studies were considered to assess the strength of evidence linking glyphosate to

non-Hodgkin lymphoma (NHL), a statistical significant association was observed in a small number of cases that was considered insufficient to conclude on the causality, due to the poor consistency of the results. Epidemiological studies face several problems linked to the small number of cancer cases and difficult identification/separation of confounders: glyphosate is generally analysed together with several other pesticides, exposure cannot be easily measured as it is generally based on interviews and questionnaires that have several intrinsic recall bias, furthermore, the classification of the type of cancer is not consistent, the adverse outcomes are not always obtained from medical records, and finally, the contribution of co-formulants' toxicity cannot be assessed. Taking into consideration the weight of evidence, EFSA concluded that there is a very limited evidence of association between glyphosate exposure and the occurrence of NHL, which would not alter the classification proposal derived from animal studies that glyphosate is unlikely to pose a carcinogenic hazard to humans.

Recognising the value that epidemiological studies can potentially provide to pesticide risk assessments, EFSA has conducted a number of initiatives in this field. First, EFSA granted an external review on epidemiological studies on pesticides which identified some limitations (Ntzani *et al.*, 2013). As a follow up, EFSA launched a project including the preparation of two Scientific Opinions, one to facilitate the use of epidemiological studies in the risk assessment of pesticides, and another investigating the potential link of pesticides toxicity with Parkinson's disease and childhood leukaemia by using criteria within the Adverse Outcome Pathway framework. The two scientific Opinions are expected to be launched for public consultation in November 2016 and June 2016 respectively.

Assessment of Plant Protection Products and co-Formulants

In line with Article 12 of Regulation (EC) No 1107/2009[2] the EFSA Conclusion presents the assessment and properties of the active substance glyphosate, considering the technical specifications provided by the applicants. The dossier includes a representative formulated product. The information on this representative product has been considered by EFSA during the evaluation of the active substance according to the relevant guidance documents for the different scientific sections. Studies on the representative product or other formulated products, either included in the dossier or made available during the peer-review process, have been considered by EFSA when relevant for the assessment of the active substance. The EFSA assessment would be relevant for the assessment of the hazard properties, including classification and labelling, and risk assessment of PPPs by the MSs, however, EFSA has not evaluated the hazard and risk of the PPPs as this is out of the EFSA remits and would require a specific mandate and submission of additional information, including the PPP dossiers.

EFSA has been mandated by the European Commission to assess a co-formulant, POE-tallowamine, that although not present in the representative formulation, is reported as co-formulant for other glyphosate containing PPPs. EFSA does not support the health-based reference values proposed by the RMS, and considers that the genotoxicity, long term toxicity/carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of this co-formulant should be clarified before setting health-based reference values and conducting the risk assessment (EFSA, 2015c).

Consumer Risk Assessment

[2] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ No L 309, 24.11.2009, p. 1-50

Following previous EU and JMPR assessments, the RMS in its initial assessment (Germany, 2013) did not propose setting an Acute Reference Dose (ARfD). However, following the re-evaluation during the EFSA peer-review, EFSA has concluded that the toxicity of glyphosate requires setting an ARfD of 0.5 mg/kg bw. It should be noted that this proposal has been agreed by the RMS.

This recommendation triggers the evaluation of acute exposure during the consumer risk assessment. EFSA will consider this conclusion during the review of the MRLs for glyphosate under Art. 12 of Regulation (EC) No 396/2005, to be conducted by EFSA in cooperation with the MSs during 2016.

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Survey of Glyphosate Residues in Honey, Corn and Soy Products

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Abstract

Samples of honey (sixty nine), pancake and corn syrup (twenty six), soy sauce (twenty eight), soy milk (eleven), and tofu (twenty) purchased in the Philadelphia, US metropolitan area were analyzed for glyphosate residue using ELISA. The limit of quantification (LOQ) and range of the method were determined for honey, pancake syrup, and corn syrup to be 15 to 800 ppb; soy sauce, soy milk, and tofu 75 to 4,000 ppb. Glyphosate residues above the limit of quantification were not found in pancake and corn syrup, soy milk, and tofu. Of the sixty-nine honey samples analyzed, forty-one samples, or fifty-nine percent (59%), had glyphosate concentrations above the method LOQ (15 ppb), with a concentration range between 17 and 163 ppb and a mean of 64 ppb. Eleven of the tested honey samples were organic; five of the organic honey samples, or forty-five percent (45%), contained glyphosate concentrations above the method LOQ, with a range of 26 to 93 ppb and a mean of 50 ppb. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62%), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb. In addition to comparison of production method (organic vs. conventional), the honey results were evaluated according to pollen source and by country of origin, grouped by GMO usage (prohibited, limited, or permitted). Glyphosate concentrations above the method LOQ (75 ppb) were also found in ten of the twenty-eight soy sauce samples evaluated (36%), with a concentration range between 88 and 564 ppb and a mean of 242 ppb; all organic soy sauce samples tested were below the method LOQ.

Keywords: Glyphosate; Honey; Honey bees; Soy sauce; ELISA

Introduction

Food consumption is an important pathway of human exposure to pesticides and other chemical contaminants. Studies have shown that exposure to contaminants in food could pose a public health risk [1,2,3]. Contaminants can enter the food supply in various ways including direct pesticide application to food crops, indirect application through the air (from drift from aerial spraying of adjacent fields), through the soil (from direct application during previous growing seasons), through the water supply (from run-off from treated areas), or through food processing (from cross-contamination from shared processing equipment) [4,5].

Glyphosate (N-(phosphonomethyl) glycine) (Figure 1), commonly sold under Monsanto's trade name Roundup®, is a non-selective herbicide that inhibits 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) in the shikimic acid pathway present in plants, bacteria, and archae [6]. EPSP synthase is the rate limiting step in the synthesis of various aromatic acids; inhibition of this enzyme results in depletion of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan [7]. Glyphosate trans locates readily in plants, making it effective for controlling perennial weeds and overwintering rhizomes and tubers. It is registered for pre planting or postharvest treatment on crops and on non-crop land [8]. Although humans do not posses the shikimic acid pathway, we are dependent upon ingested food and gut microbes, which provide essential nutrients, which do possess this pathway. Glyphosate has been patented as an antimicrobial by Monsanto Technology LLC [9], and has been shown to disrupt gut bacteria in animals [10-12]. In humans, only a small amount (~2%) of ingested glyphosate is metabolized to amino methyl phosphonic acid (AMPA), the rest enters the blood stream and is eventually eliminated through the urine [13].

The use of glyphosate in agriculture has increased significantly with the introduction of transgenic crops such as Roundup-Ready® soybeans and corn, which enable farmers to directly apply low cost broad spectrum herbicide products to their fields without harming crops. In the United States, glyphosate is currently the most widely used herbicide, with 180 to 185 millions pounds applied in the agricultural sector during 2007, 5 to 8 million pounds used in homes and gardens, and 13-15 million pounds used in industrial, commercial and governmental weed control applications [13]. The dramatic increase in the use of glyphosate in agriculture and landscape maintenance is occurring not only in the US, but throughout the world. This high level of use has led to concerns about its effects on humans and the environment. Glyphosate has traditionally been considered to be nearly non-toxic to humans [14], and therefore not problematic if ingested in food sources; as a consequence, measurement of its presence in food is very scarce [15,16]. Challenge the assertion that glyphosate is harmless, arguing that this herbicide may be a key contributor to the obesity and autism epidemics in the United States, as well as a factor in several diseases and conditions including celiac disease, Alzheimer's, Parkinson's, infertility, depression, and cancer.

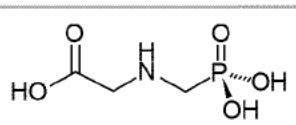

Figure 1: Glyphosate (N-(phosphonomethyl) glycine)

Glyphosate analysis in environmental and biological matrices is problematic because of its small molecular size and structural similarity to many naturally occurring plant materials such as amino acids and secondary plant compounds. It is highly soluble in water, thereby making its extraction with solvents difficult and matrix effects highly prevalent. As a result, glyphosate isolation and quantification has posed a challenge to the analytical chemist. Numerous analytical procedures have been published in the literature for the detection of this highly

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polar and amphoteric molecule [17], including gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), often coupled with mass spectrometry (MS). The co-contaminants in environmental and biological matrixes render instrumental analysis costly and time-consuming. ELISA determination, however, has allowed for the rapid, selective and sensitive determination of glyphosate [18-24].

The main objective of this survey was to obtain representative data on levels of glyphosate residues in honey, corn and pancake syrup, and soy based products, such as soy sauce, soy milk, and tofu, in products consumed by the general population in and around Philadelphia, Pennsylvania. The analytical program included the extraction of glyphosate from the various matrices and the subsequent determination of glyphosate residues by enzyme linked immune sorbent assay (ELISA).

Materials and Methods

Chemicals and reagents

Chemicals were of reagent grade and were purchased from Sigma Chemical Company, St. Louis MO, USA, except as indicated. Glyphosate (>98% purity), Chem Service, West Chester, PA, USA. Glyphosate micro titer plate ELISA, Abraxis PN 500086; Glyphosate sample diluent, PN 500082, Abraxis LLC, Warminster, PA, USA. Glyphosate stock solution was prepared in deionized water to 1.0 mg/mL; spiking solutions were prepared from the working solution using deionized water.

Samples and sample preparation/extraction

In total, 153 representative samples were purchased from markets in the Philadelphia metropolitan area (69 honey, 26 corn and pancake syrup, 28 soy sauce, 11 soy milk, and 20 tofu products).

Honey, corn and pancake syrup samples: A 0.50 g aliquot of sample was weighed into a micro centrifuge tube and 0.50 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:200 sample dilution.

Soy sauce: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Soy milk: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Tofu: A 1.0 g aliquot of sample was weighed into a 20 mL vial and 10.0 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then allowed to separate for 2 minutes. Approximately 1 mL of the mixture was transferred into a micro centrifuge tube and centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Determination of glyphosate in samples

The instructions provided in the ELISA kit user's guide were followed, in brief, glyphosate calibrators provided in the kit and the samples to be tested are derivatized for ten minutes and then added, along with an antibody specific for glyphosate to micro titer wells coated with goat anti-rabbit antibody and incubated for thirty minutes with shaking. A glyphosate horseradish peroxidase (HRP) enzyme conjugate is then added. At this point a competitive reaction occurs between the glyphosate, in the calibrators or samples, and the enzyme labeled glyphosate for the antibody binding sites on the micro titer well. The reaction is allowed to continue for sixty minutes. After a washing step an enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine) are added. The enzyme-labeled glyphosate bound to the glyphosate antibody catalyzes the conversion of the substrate /chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of diluted acid and read in a Molecular Devices micro titer plate reader (450 nm). Since the labeled glyphosate (conjugate) was in competition with the unlabeled glyphosate (sample) for the antibody sites, the color developed is inversely proportional to the concentration of glyphosate in the sample.

Data analysis

The evaluation of the assay was performed using Molecular Devices Softmax pro evaluation program (4-Parameter). The program calculates the mean absorbance value for each of the standards (B_i) and calculates the $\%B_i/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance (B_0). The program then constructs a non-linear regression model of a standard curve by plotting the $\%B_i/B_0$ for each standard on the vertical linear (y) axis versus the corresponding glyphosate concentration on the horizontal logarithmic (x) axis. The $\%B_i/B_0$ for samples is interpolated using the standard curve yielding sample concentration levels of glyphosate from the standard curve. Correlation coefficients of the assays were >0.995 and standard deviation between standard replicate analysis were < 10%.

Validation, performance and quality control

Specificity had been previously determined (ELISA user's guide), (Table 1). Recovery, limit of quantitation, range and limit of quantification were determined to test the validity of the dilution/extraction procedures of each of the matrices used in combination with the glyphosate ELISA.

COMPOUND (B/Bo)	LOD (ng/mL)	50% B/Bo (ng/mL)
Glyphosate	0.05	0.5
Glyphosine	50	3,000
Glufosinate	2,000	70,000
AMPA	35,000	>1,000,000
Glycine	>10,000	>1,000,000

Table 1: Cross-reactivity table. The reactivity of glyphosate to various related compounds expressed as LOD and as the dose required for 50% absorbance inhibition (50% B/Bo).

Results and Discussion

The method performance for glyphosate analysis was determined by conducting recovery tests on each of the matrices. To determine the accuracy of the glyphosate analysis for the sample matrices analyzed in this study, matrix samples that were glyphosate negative and positive (positive samples were not encountered with tofu, soy milk, pancake and corn syrup) were spiked as follows: 15, 40, 100, 200 and 400 ng/mL (honey, pancake and corn syrup); 75, 200, 500, 1,000 and 4,000 ng/mL [soy sauce, soy milk and tofu (ng/g)]. Analysis was performed in duplicate for all unspiked and spiked samples at all levels. Average recovery obtained for glyphosate negative honey samples fortified with glyphosate was 119 %, (SD = 10). Average recovery for glyphosate positive honey (unspiked contained 44 ng/g glyphosate) after fortification was 116 % (SD = 10). Average recovery for negative soy sauce was 94% (SD = 5), and for positive fortified soy sauce (unspiked contained 417 ng/mL) was 86% (SD = 5). The limit of quantification and range of the method were determined for honey, pancake and corn syrup to be 15 to 800 ng/g; soy sauce, soy milk, and tofu 75 to 4,000 ng/mL or ng/g, respectively.

In this study, the first sample matrix analyzed for the presence of glyphosate was honey; 69 samples were analyzed and classified into 18 groups depending on the country of origin listed on the bottles: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. The glyphosate concentrations obtained are shown in (Figure 2). Forty-one out of the sixty-nine honey samples analyzed, or fifty nine percent (59 %), had glyphosate concentrations above the method LOQ (15 ng/g) with a concentration range between 17 and 163 ng/g and a mean of 64 ng/g.

The glyphosate concentration in honey grouped by flower (pollen) source is shown in (Figure 3). The pollen types listed on the bottles were: clover (12 samples), exotic (11 samples), wildflower (11 samples), unknown (35 samples). (Figure 4) depicts the concentration of glyphosate in honey samples grouped by growing method of source pollen: organic (11 samples) and traditional (58 samples); 5 of the 11 organic samples had glyphosate concentrations above the method LOQ with a range of 26 to 93 ng/g and a mean of 50 ng /g. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62%), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb.

(Figure 5) depicts the concentration of glyphosate in honey by country and whether the use of genetically modified organisms (GMO) seeds is prohibited or permitted. The graph also shows where some minimum uses of GMO traits are allowed (Spain, and blend of Vietnam/Ukraine). The glyphosate concentration in honey originating in countries that do not allow or allow limited GMO traits (3 out of 14 samples above the LOQ) ranged from 26 to 41 ng/g with a mean of 31 ng /g. The glyphosate range for those countries that allow GMO (30 out of 43 samples above LOQ) was 21 to 163 ng/g with a mean of 71 ng /g. Samples of unknown origin (8 out of 12 samples above LOQ) ranged from 17 to 95 ng/g with a mean of 50 ng/g.

The second matrix group analyzed for glyphosate was soy sauce. The analysis consisted of 28 samples, (Figure 6). Ten out of 28 samples (36 %) had glyphosate concentrations above the method LOQ (75 ng/

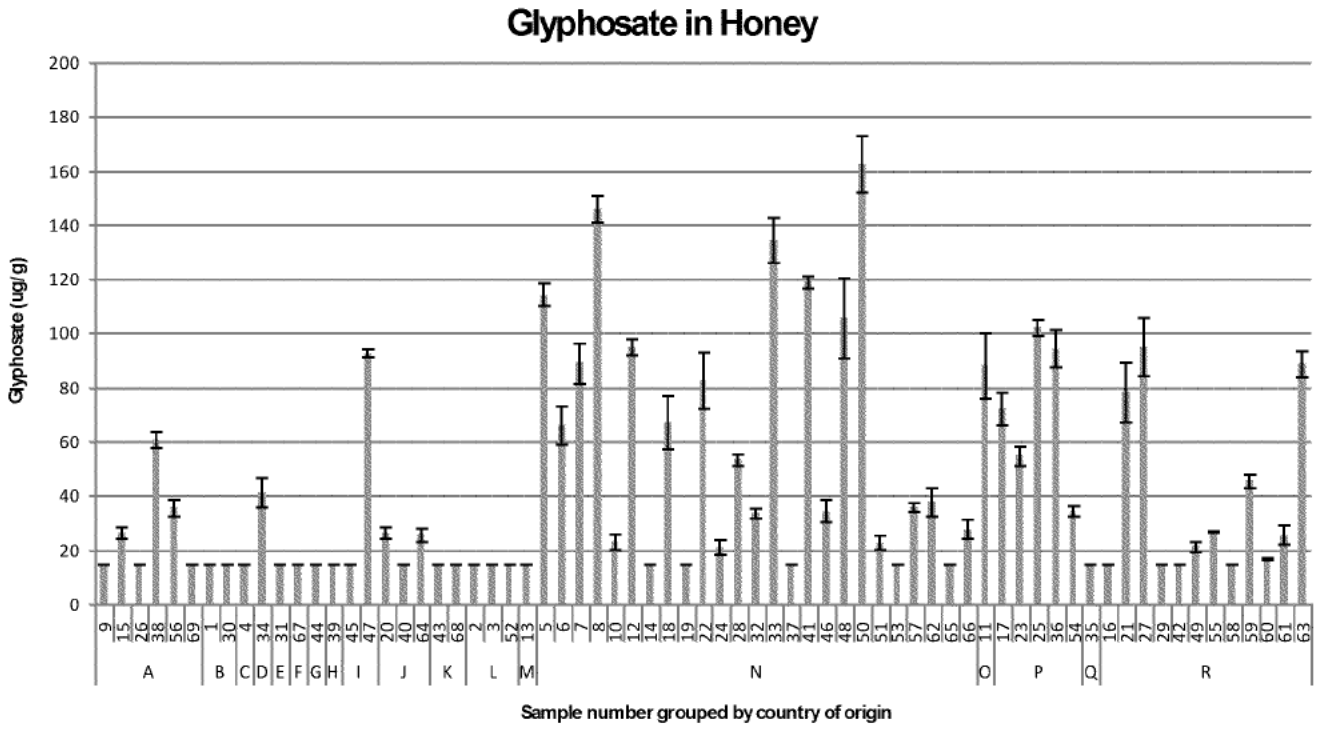

Figure 2: Concentration of glyphosate (ng/g) in honey samples listed by honey origin: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g). Error bars represent concentrations obtained during duplicate analysis.

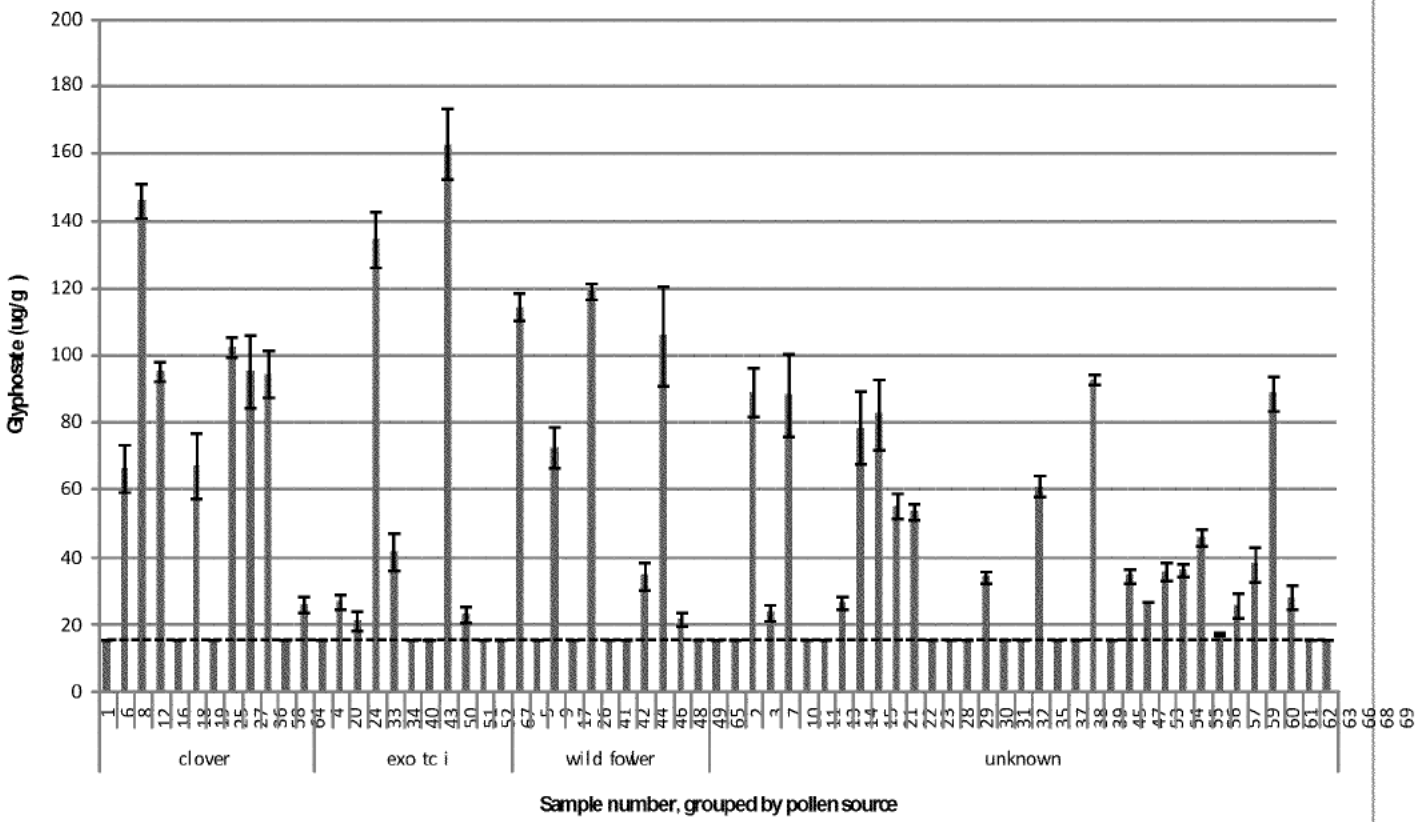

Figure 3: Concentration of glyphosate (ng/g) in honey samples by flower (pollen) source. Dashed line represents LOQ of method (15 ng/g). Exotic flowers were sophora, manuka, orange, cactus, summer flower, lychee, alfalfa, acacia.). Error bars represent concentrations obtained during duplicate analysis.

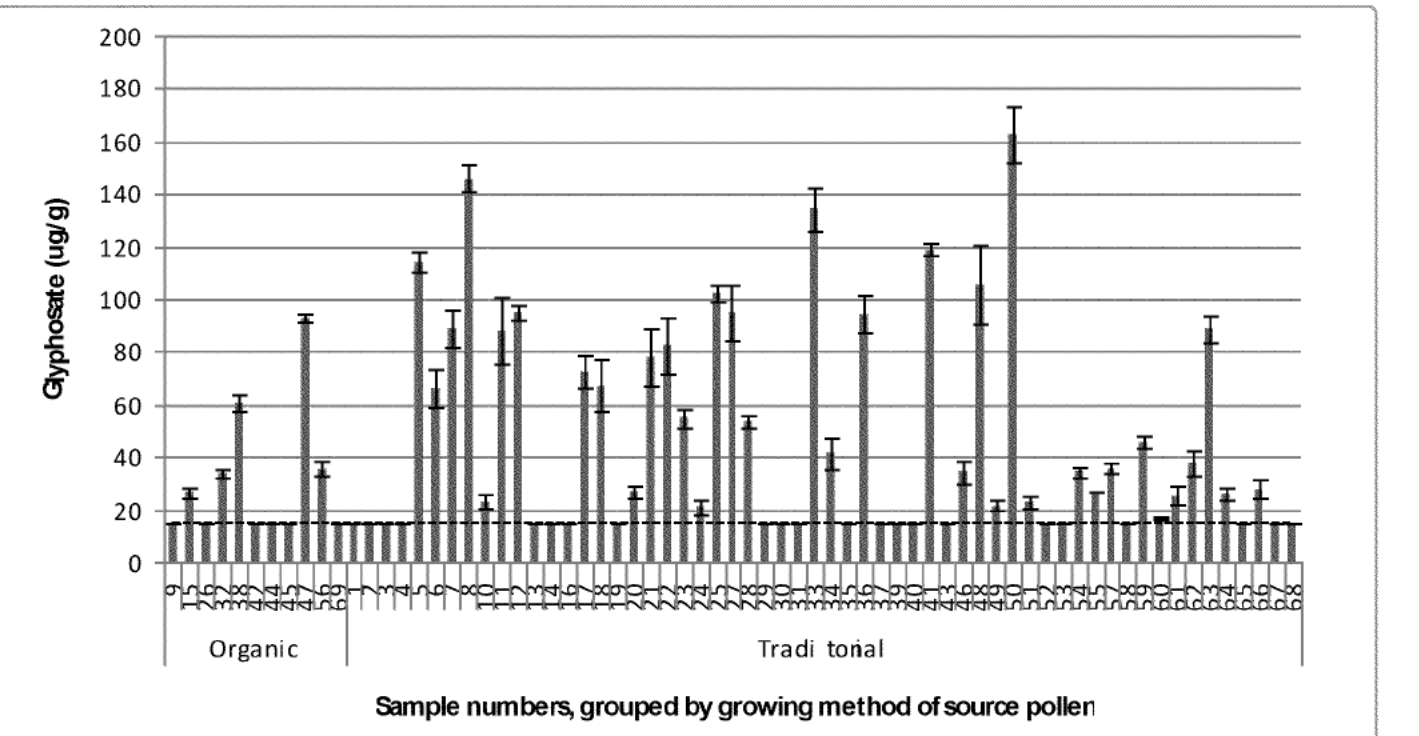

Figure 4: Concentration of glyphosate (ng/g) in honey samples by growing method of source pollen (Organic vs.Traditional). Dashed line represents LOQ of method (15 ng/g).) Error bars represent concentrations obtained during duplicate analysis.

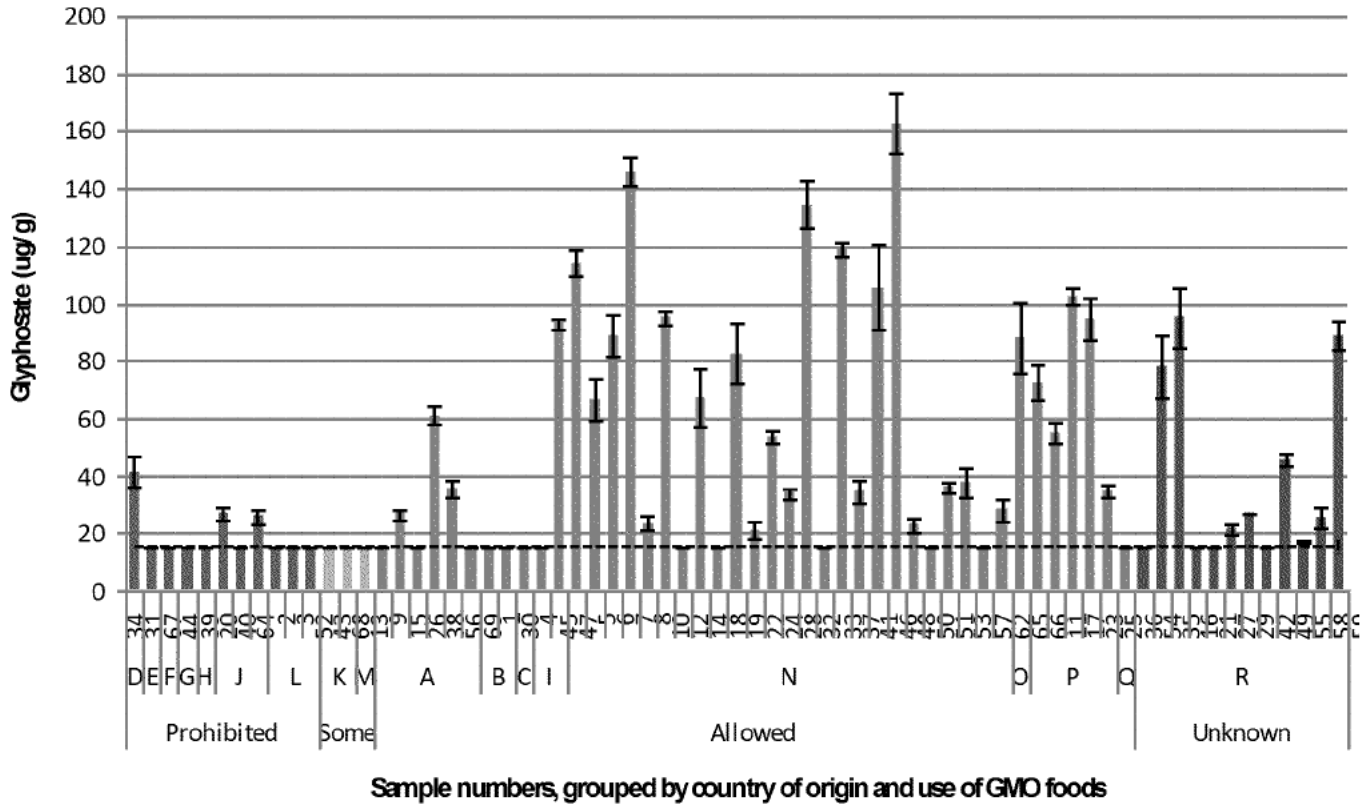

Figure 5: Concentration of glyphosate (ng/g) in honey samples listed by honey origin and the allowance of GMO use: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g).). Error bars represent concentrations obtained during duplicate analysis.

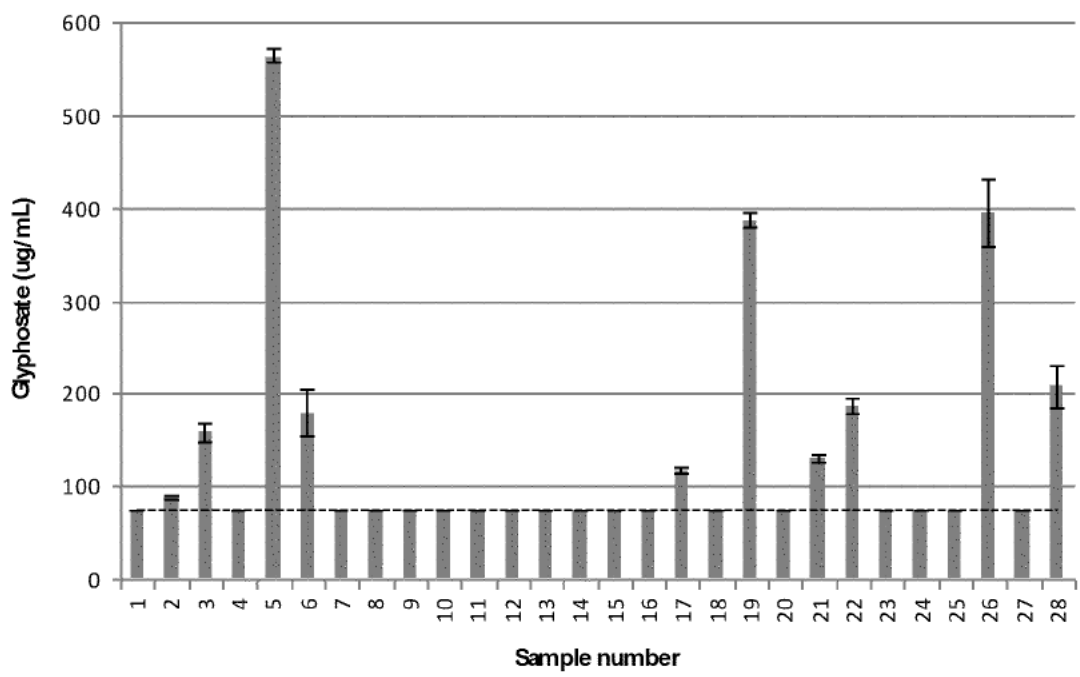

Figure 6: Concentration of glyphosate (ng/mL) in soy sauce samples. Dashed line represents LOQ of method (75 ng/mL).). Error bars represent concentrations obtained during duplicate analysis.

mL) with a concentration range between 88 and 564 ng/mL and a mean of 242 ng /mL. (Figure 7) shows the concentration of glyphosate in soy sauce by method of soy bean growing (organic vs. traditional). Therecent report from the Chinese Academy of Medical Science and the Beijing Union Hospital [20] reported an average glyphosate concentration in soy sauce of 133 ng/mL in samples that did not specify on the bottle whether or not the raw material was GM soybean. In our study, the small subset of organic labeled samples (three) was all below the limit of quantitation of the test.

Corn and pancake syrup (26 samples), soy milk (11 samples), and tofu (20 samples) tested were negative for glyphosate at the LOQ of the method (15 ng/g for pancake and corn syrup, and 75 ng/mL or ng/g for soy milk and tofu, respectively).

Studies on glyphosate residues in food are scarce. Among the few studies found was a recent report published on the incidence of glyphosate in soy sauce, conducted by the Chinese government [20]. Searches were conducted by the authors using various scientific databases on the concentration and incidence of glyphosate in honey, but these failed to provide any information. Thehoney samples analyzed in the present study show that 59 % of all samples contained glyphosate residues (ranging from 17 to 163 ng/g, mean 64 ng/g); the residue concentration does not seem to depend on pollen source or growing method, even organic honey contained glyphosate residues (5 out 11 samples, or 45 %, mean glyphosate concentration 50 ng/g). Comparing the concentration of glyphosate in honey by countries that use GMO extensively with countries that allow the use of some GMO traits and those that do not allow GMO, shows that, in general, glyphosate levels are lower in samples from countries that do not allow or allow limited use of some GMO traits, such as Spain and Vietnam/ Ukraine blend (mean 31 ng/g), compared to those countries that allow planting of GMO traits (71 ng/g). It should be noted, however, that some residues of glyphosate (although < 50 ng/g) were found in honeys originating from Germany and New Zealand, countries where no GMO planting is allowed.

TheEuropean Union has specificguidelines for the labeling of organic honey [25,26]. According to those guidelines, the location of apiaries is strictly controlled and states that "Nectar and pollen sources available over a three-kilometer radius around the apiary sites must consist essentially of organically produced crops or crops treated with low-environmental-impact methods. Apiaries must also be far enough away from any non-agricultural production source that could lead to contamination (e.g. urban centers, waste dumps, waste incinerators, etc.). Member States have the option of prohibiting the production of organic honey in certain regions or areas that do not meet these conditions. Organic honey must not contain chemicals residues (synthetic pesticides, etc.)." TheUnited States has no such guidelines for the organic production of honey, but uses organic farming certification for honey labeling purposes; one reason is that it is practically impossible to regulate without testing all honey for residues since bees can flyup to 3 miles in search of nectar and it is difficult to be certain that they do not feed on nectar contaminated by crop spraying or industrial sources. In the EU, glyphosate residues in non-organic honey regulatory limits are 50 ng/g [27], the United States does not have a limit in honey. Thelimit in drinking water in the United States is 700 ng/mL; the reference dose is 1.75 mg/Kg/day; the One-Day Health Advisory level is 20 mg/L [28]. Also, it is widely known that like milk and olive oil, honey is one of the foods that is most commonly mislabeled and adulterated [29] providing yet another source of glyphosate contamination in honeys that, according to the bottle label, originated in non-GMO countries.

Bee colony collapse disorder (CCD) is a growing threat to the efficient production of food around the world. Honey bees pollinate nearly 130 species of plant life [30], such as fruits, vegetables, nuts, and seed crops. Honeybees are therefore indirectly responsible for an

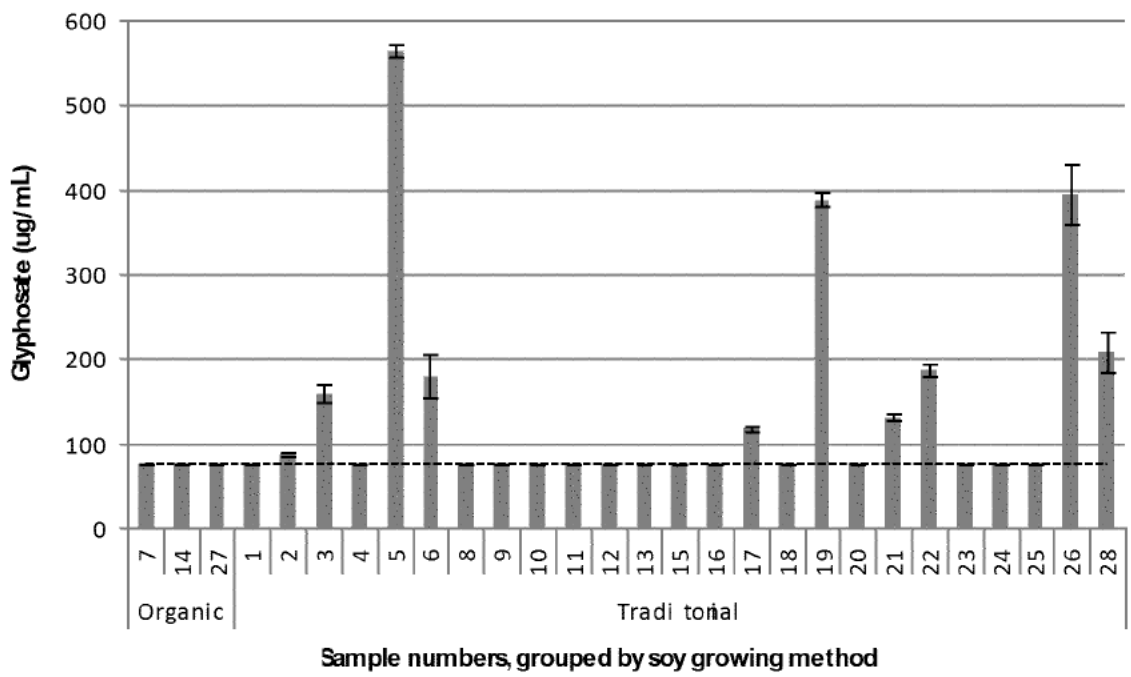

Figure 7: Concentration of glyphosate (ng/mL) in soy sauce samples by growing method of soy beans (Organic vs.Traditional). Dashed line represents LOQ of method (75 ng/mL).). Error bars represent concentrations obtained during duplicate analysis.

estimated one-third of the world food supply [31]. Although several factors are involved in CCD, including numerous pathogens and parasites, the extensive use of pesticides [32,33] such as neonicotinoids have provided evidence that these products are harmful to honey bees and have lead to a recent ban or restriction in the use of three neonicotoids by the European Union [34]. Although glyphosate is not acutely toxic to bees, it is chronically toxic to animals and is reported to disrupt the endocrine system [35,36] and a recent study indicates that honey bees exposed to increasing sub-lethal concentrations of glyphosate exhibit a decrease in acetyl cholinesterase (AChE) activity [37]. Thehigh rate of glyphosate use creates the potential for wide-spread contamination of our food chain. Glyphosate is used throughout the bee foraging period in high amounts and is found in the air, water, and in plant parts frequented by bees, such as flowers and buds, potentially contaminating the nectar collected by bees from contaminated plants [38]. Based on its prevalence in the environment, as well as our findingsin honey samples, we propose that future studies should be conducted to determine if glyphosate is in fact a contributing factor in CCD.

Conclusion

Thistudy indicates the presence of glyphosate residues in honey and soy sauce, but not in pancake and corn syrups or soy based products such as soy milk and tofu. Forty one out of sixty nine (59%) honey samples analyzed contained glyphosate at a concentration above the method LOQ (15 ng/g) with a range between 17-163 ng/g and a mean of 64 ng/g. Ten out of twenty eight (36%) soy sauce samples contained glyphosate at a concentration above the method LOQ (75 ng/mL) with a range between 88-564 ng /mL and a mean of 242 ng /mL. Future studies should be conducted on many other food products to determine the extent of glyphosate residue contamination.

Acknowledgements

We would like to give our thanks to Erin Faltin for the preparation of the graphs presented in this paper.

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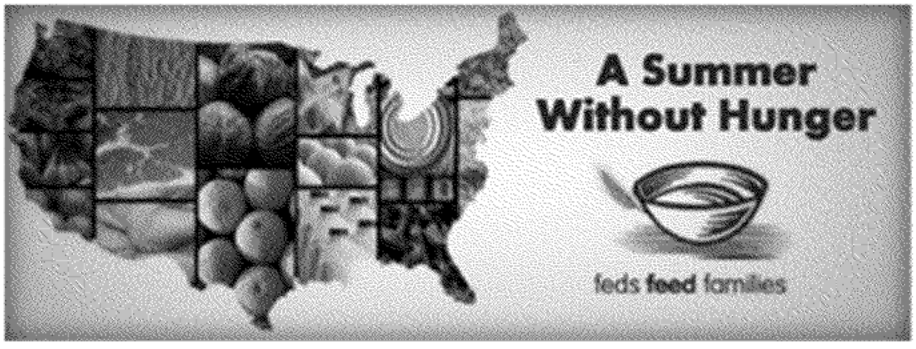
Citation: Rubio F, Guo E, Kamp L (2014) Survey of Glyphosate Residues in Honey, Corn and Soy Products. J Environ Anal Toxicol 4: 249. doi: 10.4172/2161-0525.1000249

To: Councell, Terry[Terry.Councell@fda.hhs.gov]
Cc: Miller, David[Miller.DavidJ@epa.gov]
From: Hrdy, David
Sent: Tue 11/10/2015 7:47:53 PM
Subject: RE: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

(b) (5)

Hrdy



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Senior Scientist
CEB/HED/OPP/OCSPP
US EPA www.epa.gov/pesticides
Mailcode 7509P
Telephone: **703.305.6990**
Fax: 703.305.5147
OFFICE 10248 Potomac Yard 1 (South)
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From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, November 10, 2015 2:31 PM
To: Miller, David <Miller.DavidJ@epa.gov>; Hrdy, David <Hrdy.David@epa.gov>
Subject: FW: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society
~~~

FYI

From: Chamkasem, Narong
Sent: Tuesday, November 10, 2015 2:24 PM
To: Sack, Chris A; Councell, Terry; Yeung, Andrew; Podhorniak.Lynda@epa.gov; Islam, Mohammed R
Cc: Harmon, Tiffany M
Subject: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

http://naturalsociety.com/researchers-discover-glyphosate-herbicide-honey-soy-sauce/

To: Miller, David[Miller.DavidJ@epa.gov]; Hrdy, David[Hrdy.David@epa.gov]
From: Councell, Terry
Sent: Tue 11/10/2015 7:30:42 PM
Subject: FW: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

FYI

From: Chamkasem, Narong
Sent: Tuesday, November 10, 2015 2:24 PM
To: Sack, Chris A; Councell, Terry; Yeung, Andrew; Podhorniak.Lynda@epa.gov; Islam, Mohammed R
Cc: Harmon, Tiffany M
Subject: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

http://naturalsociety.com/researchers-discover-glyphosate-herbicide-honey-soy-sauce/

To: Miller, David[Miller.DavidJ@epa.gov]
From: Councell, Terry
Sent: Tue 11/3/2015 1:50:25 PM
Subject: RE: Glyphosate sampling
Import Alerts.doc

David

Attached are some of the import alert data from the EU. I included all the import alerts as it contains olive oils, other dried fruits in addition to juice concentrates. I highlighted in red the juice concentrates. It looks like Germany and Netherlands are getting the lions share. FDA puts the import alerts on the web at http://www.accessdata.fda.gov/cms_ia/countrylist.html. They are usually current within 3 months.

Terry

-----Original Message-----
From: Miller, David [mailto:Miller.DavidJ@epa.gov]
Sent: Tuesday, November 03, 2015 8:28 AM
To: Haynes, Diana - AMS; Councell, Terry
Cc: Hrdy, David; Nako, Steve; Smith, Charles; Metzger, Michael
Subject: FW: Glyphosate sampling

(b) (5)

David.

-----Original Message-----
From: Miller, David
Sent: Tuesday, November 03, 2015 8:23 AM
To: Vogel, Dana <Vogel.Dana@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>
Subject: RE: Glyphosate sampling

(b) (5)

David.

-----Original Message-----
From: Vogel, Dana

Sent: Tuesday, November 03, 2015 7:44 AM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: Glyphosate sampling

(b) (5)

Dana Vogel
Sent from my iPhone

Biosearch S.A.
Date Published : 07/15/2011

Poligono Industrial Alcantarilla, 0 S/N , Talayuela, Caceres SPAIN
54 K - - 03 Valerian (Herbal & Botanicals, not Teas II)
Date Published: 07/15/2011

Desc:Valeriana (Dry Extract)
Notes:693603 - NYK-DO Mfr. per product label
Problems: CHLORPYRIFOS;

Biosearch S.A.
Date Published : 07/15/2011

Camino De Punchil 66 , Granada, SPAIN
54 K - - 03 Valerian (Herbal & Botanicals, not Teas II)
Date Published: 07/15/2011

Desc:Valeriana (Dry Extract)
Notes:693603 NYK-DO
Problems: CHLORPYRIFOS;

Cesma, SL
Date Published : 08/01/2011

Poligono Industrial La Alberca, 5 , Guarena, ES-BA SPAIN
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 08/01/2011

Desc:Olive Oil
Notes:690852 SWID
Problems: TEBUCONAZOLE;

Covides SCCL
Date Published : 06/07/2013

45, Rambla De Nostra Senyora , Vilafranca Del Penedes, Barcelona SPAIN
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 06/07/2013

Desc:Olive Oil
Notes:Sample #807183 PRL/NW
Problems: CHLORPYRIFOS;

Eckes Granini Iberica S.A.
Date Published : 09/16/2009

Travessera De Gracia 73 79 3-1 , Barcelona, SPAIN
21 K - - 13 Peach, Juice, Pit Fruit Juices or Concentrates
Date Published: 09/16/2009

Notes:6/16/04
Problems: PROCYMIDONE;

Hdos. De Pepe El Bueno S.L.
Date Published : 09/16/2009

Carreterra General No. 18 , Asturias, SPAIN
24 B - - 05 Fava Bean, Dried or Paste
Date Published: 09/16/2009

Desc:beans; white;
Notes:6/13/03
Problems: PROCYMIDONE;
24 B - - 07 Kidney Bean, Dried or paste
Date Published: 09/16/2009

Desc:beans; white;
Notes:6/13/03
Problems: PROCYMIDONE;
24 B - - 10 Navy (White) Beans (Baked Beans, Pork & Beans), Dried or Paste
Date Published: 09/16/2009

Desc:beans; white;
Notes:6/13/03
Problems: PROCYMIDONE;
24 B - - 99 Bean, Corn, Pea, Dried or Paste, N.E.C. (Vegetable)
Date Published: 09/16/2009

Desc:beans; white;
Notes:6/13/03
Problems: PROCYMIDONE;

La Masrojana S.L.
Date Published : 04/25/2014

av 11 de setembre 2 , El Masroig, TARRAGONA SPAIN
21 G - - 11 Olives, Green, (Pit Fruit)
Date Published: 04/25/2014

Desc:Arequina Olives
Problems: CHLORPYRIFOS;

MKOLIVEOILCO S.L.
Date Published : 11/18/2014

Edificio MK, Carretera Puente Genil-Este , Km 10.300, Apartado 211 , Puente Genil, Cordoba SPAIN
26 B - - 99 Vegetable Oil Seed Stock, N.E.C.
Date Published: 11/18/2014

Desc:Grape Seed Oil
Problems: CHLORPYRIFOS;
26 C - - 99 Refined Vegetable Oil, Single Ingredient, N.E.C.
Date Published: 11/18/2014

Desc:Grape Seed Oil
Problems: CHLORPYRIFOS;

Olis de Catalunya, S.L.
Date Published : 03/27/2014

Carretera de la Selva del Camp km 1,6 , Vilallonga Del Camp, Tarragona SPAIN
26 A - - 04 Olive Oil, Crude (for Refining)
Date Published: 03/27/2014

Desc:Extra Virgin Olive Oil
Notes:842868 SWID
Problems: CHLORPYRIFOS;
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 03/27/2014

Desc:Extra Virgin Olive Oil
Notes:842868 SWID
Problems: CHLORPYRIFOS;
26 B - - 99 Vegetable Oil Seed Stock, N.E.C.
Date Published: 03/27/2014

Desc:Grape Seed Oil
Problems: CHLORPYRIFOS;

Olis de Catalunya, S.L.
Date Published : 03/27/2014

Carretera de la Selva del Camp km 1,6 , Vilallonga Del Camp, Tarragona SPAIN
26 A - - 04 Olive Oil, Crude (for Refining)
Date Published: 03/27/2014

Desc:Extra Virgin Olive Oil
Notes:842868 SWID
Problems: CHLORPYRIFOS;
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 03/27/2014

Desc:Extra Virgin Olive Oil
Notes:842868 SWID
Problems: CHLORPYRIFOS;
26 B - - 99 Vegetable Oil Seed Stock, N.E.C.
Date Published: 03/27/2014

Desc:Grape Seed Oil
Problems: CHLORPYRIFOS;

REFINERIA ANDALUZA, S.A.
Date Published : 12/23/2013

CTRA. N-IV, KM. 389 , Alcolea, Cordoba SPAIN
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 12/23/2013

Desc:Olive Oil
Notes:838476 SEA-DO
Problems: TERBUTHYLAZINE;

Vecomar Alimentacion, S.L.
Date Published : 09/16/2009

Pol. Ind. San Jorge , Las Torres De Cotilla (Murcia), SPAIN
24 F - E 07 Pepper, Sweet (Fruit Used as Vegetable)
Date Published: 09/16/2009

Desc:pimentos sweet peppers
Notes:10/16/03
Problems: METHIOCARB;
24 F - J 07 Pepper, Sweet (Fruit Used as Vegetable)
Date Published: 09/16/2009

Desc:Pimentos
Notes:10/16/03
Problems: METHIOCARB;

Vicente Bravo, S.L.
Date Published : 08/22/2011

Avda Salzillo P. 21/24 / Pol-Ind Oeste , P.O. Box 238 , San Gines, Murcia SPAIN
28 B - - 22 Lemon, Ground, Cracked (Spice)
Date Published: 08/22/2011

Desc:Lemon Peel
Notes:629262 NYK-DO;
Problems: PROCHLORAZ;

GREECE

ARGO PIRAEUS
Date Published : 10/08/2013

25th Martiou 106 Keratsini , Piraeus, GR-A1 GREECE
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 10/08/2013

Desc:Olive Oil
Problems: CHLORPYRIFOS;
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 10/08/2013

Desc:Olive Oil
Problems: CHLORPYRIFOS;

G A Gotos
Date Published : 09/16/2009

Unknown Street , Athens, GREECE

21 G - - 11 Olives, Green, (Pit Fruit)
Date Published: 09/16/2009

Desc:Mariana Brand
Notes:9/15/95; PACKER
Problems: CHLORPYRIFOS;

Ilis Exporting Co
Date Published : 09/16/2009

Unknown , Havari, GREECE
24 F - - 99 Fruits Used as Vegetables, N.E.C.
Date Published: 09/16/2009

Desc:Greek Golden Peppers
Notes:11/5/91
Problems: PYRAZOPHOS(AFUGAN);

Mani Imports-Exports
Date Published : 09/16/2009

2 Par. Ayras 10 , Kalamata, GREECE
21 G - - 11 Olives, Green, (Pit Fruit)
Date Published: 09/16/2009

Desc:cracked green olives
Notes:11/2/92
Problems: CHLORPYRIFOS;

Nicoglou Bakery Yeast Sa
Date Published : 09/16/2009

17 Joan Koletti Street , Thessaloniki, GREECE
24 T - - 99 Leaf & Stem Vegetables, N.E.C.
Date Published: 09/16/2009

Desc:grape leaves stuffed with rice
Notes:3/7/94
Problems: BROMOPROPYLATE;

Olympion
Date Published : 05/08/2015

Skinto - Havari , Chavari, Ileias GREECE
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 05/08/2015

Desc:Olive Oil
Problems: CHLORPYRIFOS;
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 05/08/2015

Desc:Olive Oil
Problems: CHLORPYRIFOS;

Onassis Greek Tinned Food Industry
Date Published : 09/16/2009

N. Magnisia Berias 16 Sts. , Thessaloniki, GREECE
24 T - - 99 Leaf & Stem Vegetables, N.E.C.
Date Published: 09/16/2009

Desc:stuffed grape leaves
Notes:4/21/94
Problems: BROMOPROPYLATE;

ITALY

Az. Agr. La Gallinella
Date Published : 02/11/2015

Via Semiana 1 , Velezzo Lomellina, PV ITALY
02 A - - 06 Rice, Wild, Whole Grain
Date Published: 02/11/2015

Desc:Black Rice
Problems: PIRIMIPHOS-METHYL;

Azienda Agricola Ferrante Michele
Date Published : 11/25/2013

Via degli Orti,2 , Controne, SA ITALY
24 H - - 50 Tomato, Dried
Date Published: 11/25/2013

Desc:Sundried Cherry Tomatoes
Notes:833811
Problems: TOLCLOFOS METHYL;

BRUNELLA FOOD INDUSTRY s.r.l.
Date Published : 02/26/2013

VIA TERMINE BIANCO n.42 , San Marzano Sul Sarno, SA ITALY
24 A - - 70 Lentils

Date Published: 02/26/2013

Desc:Canned Lentils
Notes:774288
Problems: PIRIMIPHOS-METHYL;

EPO Estratti Piante Officinali S.r.l.
Date Published : 07/16/2013

Via Stadera 19 , Milano, Mi ITALY
54 E - - 98 Bilberry (Herbal & Botanical Teas)
Date Published: 07/16/2013

Desc:Bilberry Extract
Notes:807623 NYK-DO
Problems: CHLORPYRIFOS;
54 F - - 98 Bilberry (Herbal & Botanicals, not Teas)
Date Published: 07/16/2013

Desc:Bilberry Extract
Notes:807623 NYK-DO
Problems: CHLORPYRIFOS;

Epo Srl
Date Published : 07/16/2013

Via Friuli S N , Buccinasco, Mi ITALY
54 E - - 98 Bilberry (Herbal & Botanical Teas)
Date Published: 07/16/2013

Desc:Bilberry Extract
Notes:807623 NYK-DO
Problems: CHLORPYRIFOS;
54 F - - 98 Bilberry (Herbal & Botanicals, not Teas)
Date Published: 07/16/2013

Desc:Bilberry Extract
Notes:807623 NYK-DO
Problems: CHLORPYRIFOS;

Eureka S.R.L.
Date Published : 09/16/2009

Via C Battisti 16 , Acerra Napoli, ITALY
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 09/16/2009

Notes:5/28/08
Problems: ENDOSULFAN; SEE 900, 901, 902;
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 09/16/2009

Notes:5/28/08
Problems: ENDOSULFAN; SEE 900, 901, 902;

Euricom S.P.A
Date Published : 10/02/2009

Via Stazione 119 , Valle Lomellina, PV ITALY
02 - - - - Whole Grain/Milled Grain Prod/Starch
Date Published: 10/02/2009

Notes:4/08/09
Problems: PIRIMIPHOS-METHYL;

Eurofruit S.r.l.
Date Published : 09/16/2009

Via Vittorio, Emanuele 3 , Mugnano del Cardinale, Zona Industriale , Avella, It-Av ITALY
21 G - - 03 Cherry Fruit (Pit Fruit)
Date Published: 09/16/2009

Desc:cherries in SO2
Notes:5/18/04
Problems: PROCYMIDONE;

European Rice Company
Date Published : 09/16/2009

Via Bassano 13 , Vercelli, ITALY
02 D - - 02 Rice, Plain (White or Polished) Processed (Packaged)
Date Published: 09/16/2009

Desc:Arborio rice
Notes:3/17/04
Problems: DELTAMETHRIN;

Felice Bonardi
Date Published : 09/16/2009

Corso Plave, 57 , Alba, CN ITALY
32 B - - 99 Wines, N.E.C.
Date Published: 09/16/2009

Desc:Felice Bonardi Brand Asti Spumante (vintage yr assumed 1990)
Notes:4/2/91
Problems: PROCYMIDONE;

Frantoi Cutrera Di Cutrera Giovanni e C.
Date Published : 04/01/2015

Contrada Piano dell'Acqua, 71 , Chiaramonte Gulfi, RG ITALY
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 04/01/2015

Desc:Olive Oil
Problems: CHLORPYRIFOS; PHOSMET;
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 04/01/2015

Desc:Olive Oil
Problems: CHLORPYRIFOS; PHOSMET;

L'Aromatica Aquilana Srl
Date Published : 09/16/2009

Via Gorbaldi 63 , Corugalo, Milan ITALY
24 F - - 50 Tomatoes (Fruit Used as Vegetable)
Date Published: 09/16/2009

Desc:dried tomatoes
Notes:1/10/91
Problems: PIRIMIPHOS-METHYL;

Oleificio Borelli Spa
Date Published : 06/23/2014

Via Nazionale 36 , Pontedassio, IM ITALY
24 F - - 50 Tomatoes (Fruit Used as Vegetable)
Date Published: 06/23/2014

Desc:Canned Whole Peeled Tomatoes
Problems: CHLORPYRIFOS;

Oleificio Pasquinoni S.N.C.
Date Published : 04/02/2013

Via T. Tasso, 3 SS. San Marino Km 7,5 , Cerasolo Ausa Di Coriano, IT-RN ITALY
26 A - - 09 Virgin Olive Oil, Crude
Date Published: 04/02/2013

Desc:Olive Oil
Notes:Entry; 112-1417982-8
Problems: CHLORPYRIFOS;
26 C - - 04 Olive Oil, Refined, Single Ingredient
Date Published: 04/02/2013

Desc:Olive Oil
Notes:Entry; 112-1417982-8
Problems: CHLORPYRIFOS;

Pastificio Gallo Natale E. F. LLI
Date Published : 10/28/2014

Via Albert Einstein 3 , Mazara Del Vallo, TP ITALY
04 A - - 05 Spaghetti
Date Published: 10/28/2014

Desc:Fettuccine
Problems: PIRIMIPHOS-METHYL;

Riso Gallo S.p.A
Date Published : 02/05/2014

Viale Riccardo Preve n. 4 , Robbio, PV ITALY
02 A - - 05 Rice, Cultivated, Whole Grain
Date Published: 02/05/2014

Desc:ITALIAN ARBORIO Risotto Rice
Notes:AQZ-0334082-8; 820534-LOS
Problems: PIRIMIPHOS-METHYL;

GERMANY

Doehler Neuenkirchen GmbH
Date Published : 06/20/2013

Dorfstrasse17 , D-21763 , Neuenkirchen, DE-NI GERMANY
20 D - - 07 Elderberry Juice, Berry Juice or Concentrates
Date Published: 06/20/2013

Desc:Elderberry Juice
Notes:807389 SWID; Pesticides; THPI

Haelssen & Lyon GmbH
Date Published : 12/22/2011

Pickhuben 9 , Hamburg, Hamburg GERMANY
20 B - - 24 Wolfberry (Chinese or Southeast Asian berry of the Chinese matrimony vine, w/slight licorice flavor)
Date Published: 12/22/2011

Desc:Gogi Berries Wolfberries
Notes:722915 BLT-DO
Problems: ACETAMIPRID; CARBENDAZIM (MBC); CARBOFURAN; TRIAZOPHOS; PROPARGITE; PYRIDABEN; THIOPHANATE-METHYL; CLOFENTEZINE;

Interquell Staerkechemie GMBH
Date Published : 09/16/2009

Bahnhofstr 94 , Gro?aitingen, GERMANY
24 H - - 50 Tomato, Dried
Date Published: 09/16/2009

Desc:dried tomato flakes
Notes:10/10/03
Problems: BROMOPROPYLATE;

Krauter Mix GmbH
Date Published : 09/04/2013

Wiesentheider Str. 4 , Abtswind, GERMANY
54 J - - 03 Valerian (Herbal & Botanicals II)
Date Published: 09/04/2013

Desc:Valerian root powder valerian root cut
Notes:Sample # 819582; 819583;p;p'-DDE
Problems: CHLORPYRIFOS;

Martin Bauer Gmbh & Co. KG
Date Published : 10/23/2013

5-7 Dutendorfer Strasse , Vestenbergsgreuth, GERMANY
31 P - - 04 Tea, Camomille, Chamomile (Tea Substitute)
Date Published: 10/23/2013

Desc:Chamomile Pellets
Notes:Sample #808840 LOS-DO
Problems: CARBENDAZIM (MBC); DIFENOCONAZOLE; LINURON; PIPERONYL BUTOXIDE;
54 F - - 04 Camomille (Herbal & Botanicals, not Teas)
Date Published: 10/23/2013

Desc:Chamomile Pellets
Notes:Sample #808840 LOS-DO
Problems: CARBENDAZIM (MBC); DIFENOCONAZOLE; LINURON; PIPERONYL BUTOXIDE;

Mueggenburg Pflanzliche Rohstoffe GmbH & Co KG
Date Published : 05/08/2015

Tegelbarg 35 , Bad Bramstedt, Schleswig-holstein GERMANY
25 S - - 04 Mushroom, Shiitake, Fungi Products, N.E.C.
Date Published: 05/08/2015

Desc:Shitake Mushroom Powder
Problems: CARBENDAZIM (MBC);
54 F - - 99 Herbals & Botanicals (not Teas), N.E.C.
Date Published: 05/08/2015

Desc:Shitake Mushroom Powder
Problems: CARBENDAZIM (MBC);

NORDFROST Kuehl- und Lagerhaus GmbH & Co. KG
Date Published : 09/16/2009

Bredowstr. 21 , Hamburg, De-Hh GERMANY
20 A - D 01 Blackberries (Berry)
Date Published: 09/16/2009

Notes:12/22/04
Problems: VINCLOZOLIN;
20 A - T 01 Blackberries (Berry)
Date Published: 09/16/2009

Notes:12/22/04
Problems: VINCLOZOLIN;

Paradiesfrucht GmbH
Date Published : 01/12/2010

Bergener Str. 10 , Salzwedel, Sachsen-Anhalt GERMANY
20 A - - 01 Blackberries (Berry)
Date Published: 02/06/2012

Desc:Blackberries
Notes:727864 MIN-DO
Problems: PROCYMIDONE;
20 B - - 01 Blackberries, Dried or Paste
Date Published: 02/06/2012

Desc:Blackberries
Notes:727864 MIN-DO
Problems: PROCYMIDONE;
20 B - - 13 Raspberries, Red, Dried or Paste
Date Published: 03/24/2011

Desc:Freeze Dried Raspberry Slices
Notes:671381 SEA; Carbendazim
20 B - - 14 Strawberries, Dried or Paste
Date Published: 11/09/2012

Desc:Strawberries freeze dried
Notes:777938 NWE-DO
Problems: TETRADIFON;
20 B - - 99 Berries, Dried or Paste, N.E.C.
Date Published: 02/06/2012

Desc:Blackberries
Notes:727864 MIN-DO
Problems: PROCYMIDONE;

W. Schumacher & Co. GmbH
Date Published : 09/16/2009

Roentgenstrasse 3 , Wittlich, Rheinland-Pfalz GERMANY
24 T - H 11 Celery (Leaf & Stem Vegetable)
Date Published: 09/16/2009

Notes:1/19/07
Problems: FENVALERATE;
24 U - - 11 Celery, Dried or Paste
Date Published: 09/16/2009

Notes:1/19/07;8/14/07 dehydrated celery stalk and leaves granules
Problems: FENVALERATE; DIFENOCONAZOLE;
24 U - - 18 Kale, Dried or Paste
Date Published: 06/20/2013

Desc:Kale Dried
Notes:Entry #112-2430185-9; I-cyhalothrin
Problems: CARBENDAZIM (MBC); CHLORFENAPYR;
25 J - - 04 Scallions, Green Onions (Root & Tuber Vegetable)
Date Published: 06/14/2012

Desc:Scallion Green Onion
Notes:756164 NOL-DO;Propamocarb
25 P - - 03 Mushroom, Straw, Whole (Button) (Fungi)

Date Published: 10/16/2014

Desc:Dehydrated Mushrooms 6-10mm
Problems: THIOPHANATE-METHYL; PROCHLORAZ; CARBOFURAN; DIETHOFENCARB;
25 P - - 99 Mushrooms and Other Fungi Products, Whole (button), N.E.C.
Date Published: 10/16/2014

Desc:Dehydrated Mushrooms 6-10mm
Problems: DIETHOFENCARB; PROCHLORAZ; THIOPHANATE-METHYL; CARBOFURAN;

UNITED KINGDOM

ECO Dehydrates & Essential Oils
Date Published : 09/16/2009

Unknown , Lincolnshire, UNITED KINGDOM
24 U - - 25 Spinach, Dried or Paste
Date Published: 09/16/2009

Desc:dehydrated spinach powder
Notes:9/12/91
Problems: DICOFOL; SEE 253, 254;

F G S Mills Kishan House
Date Published : 09/16/2009

24-44 Burfield Street , Leicester, UNITED KINGDOM
02 E - - 99 Wheat Flour, N.E.C.
Date Published: 09/16/2009

Desc:Bajri flour
Notes:2/1/94
Problems: BHC; SEE 903, 904, 905, 050;

MACKAYS LTD
Date Published : 03/09/2011

21-23 Thistle Street , CARNOUSTIE, UNITED KINGDOM
20 C - - 13 Raspberry, Red, Jam, Jelly, Preserves, Marmalade, Butter and Candied
Date Published: 03/09/2011

Desc:Raspberry Jam
Notes:654880 LOS-DO
Problems: PYRIMETHANIL;
20 C - - 99 Berries, Jam, Jelly, Preserves, Marmalade, Butter and Candied, N.E.C.
Date Published: 03/09/2011

Desc:Raspberry Jam
Notes:654880 LOS-DO
Problems: PYRIMETHANIL;

MACKAYS LTD
Date Published : 03/09/2011

21-23 Thistle Street , CARNOUSTIE, UNITED KINGDOM
20 C - - 13 Raspberry, Red, Jam, Jelly, Preserves, Marmalade, Butter and Candied
Date Published: 03/09/2011

Desc:Rasberry Jam
Notes:654880 LOS-DO
Problems: PYRIMETHANIL;
20 C - - 99 Berries, Jam, Jelly, Preserves, Marmalade, Butter and Candied, N.E.C.
Date Published: 03/09/2011

Desc:Rasberry Jam
Notes:654880 LOS-DO
Problems: PYRIMETHANIL;

Mackays Ltd
Date Published : 03/09/2011

James Chalmers Road , Kirkton Industrial Estate , Arbroath, UNITED KINGDOM
20 C - - 13 Raspberry, Red, Jam, Jelly, Preserves, Marmalade, Butter and Candied
Date Published: 03/09/2011

Desc:Rasberry Jam
Notes:654880 LOS-DO
Problems: PYRIMETHANIL;
20 C - - 14 Strawberry, Jam, Jelly, Preserves, Marmalade, Butter and Candied
Date Published: 12/30/2014

Desc:Strawberry preserve with champagne
Problems: BUPIRIMATE; PIRIMICARB;
20 C - - 99 Berries, Jam, Jelly, Preserves, Marmalade, Butter and Candied, N.E.C.
Date Published: 01/20/2015

Desc:Scottish Three Berry Preserve (Strawberry; Raspberry; Blackcurrant)
Problems: PIRIMICARB;
20 C - - 99 Berries, Jam, Jelly, Preserves, Marmalade, Butter and Candied, N.E.C.
Date Published: 03/09/2011

Desc:Rasberry Jam
Notes:654880 LOS-DO

Problems: PYRIMETHANIL;

NETHERLANDS

ADM Cocoa B.V.
Date Published : 06/25/2015

Stationsstraat 76 , Koog aan de Zaan, NETHERLANDS
34 B - - 08 Medium Fat Cocoa (except Chocolate Candy and Chocolate Beverage Base)
Date Published: 09/30/2015

Desc:Cocoa Powder
Problems: METALAXYL;
34 B - - 14 Cocoa (except Chocolate Candy and Chocolate Beverage Base)
Date Published: 06/25/2015

Desc:Cocoa Powder
Problems: METALAXYL;

Adm Cocoa
Date Published : 08/11/2015

Portier Hoogstraat 37 , Koog Aan De Zaa, NETHERLANDS
34 B - - 08 Medium Fat Cocoa (except Chocolate Candy and Chocolate Beverage Base)
Date Published: 09/30/2015

Desc:Cocoa Powder
Problems: METALAXYL;
34 B - - 14 Cocoa (except Chocolate Candy and Chocolate Beverage Base)
Date Published: 08/11/2015

Desc:Cocoa Powder
Problems: METALAXYL;

DiPasa Europe B V
Date Published : 09/16/2009

Marsteden 56 , Enschede, NETHERLANDS
23 K - - 02 Sesame Seed (Edible)
Date Published: 09/16/2009

Notes:12/2/94
Problems: BHC; SEE 903, 904, 905, 050;

Doens Food Ingredients B.V.
Date Published : 06/10/2013

Oranjestraat 40a , Ijzendijke, Zeeland NETHERLANDS
02 D - - 07 Rice, Basmati, Processed (Packaged)
Date Published: 06/10/2013

Desc:Basmati Rice
Problems: CHLORPYRIFOS;

Oerlemans Foods Nederland Bv
Date Published : 09/16/2009

Archimedesweg 19 , P.O. Box 3351-5902 , Venlo, NETHERLANDS
20 A - D 14 Strawberries (Berry)
Date Published: 09/16/2009

Notes:9/26/02
Problems: PROCYMIDONE; TOLYLFLUANID;
20 A - T 14 Strawberries (Berry)
Date Published: 09/16/2009

Notes:9/26/02
Problems: PROCYMIDONE; TOLYLFLUANID;

SVZ International BV
Date Published : 09/16/2009

Oude Kerkstraat 8 , Etten-leur, Noord-Brabant NETHERLANDS
20 A - T 05 Currants (Berry)
Date Published: 09/16/2009

Desc:black currants (boiled; frozen in cubes)
Notes:6/6/2000
Problems: TOLYLFLUANID;
20 D - - 05 Currant Juice, Berry Juice or Concentrates
Date Published: 09/16/2009

Desc:black currant juice concentrate
Notes:7/2/07 (tetrahydrophthalimide per FACTS summary report)
25 J - T 25 Onion Bulb (Yellow, White, Red, Etc.) (Root & Tuber Vegetable)
Date Published: 09/16/2009

Desc:frozen onion (silverskin)
Notes:2/29/2000
Problems: PROCYMIDONE;

Sonder Jansen Bv
Date Published : 05/27/2014

Dorpsstraat 66 , Rosmalen, Noord-brabant NETHERLANDS
20 D - - 02 Blueberry Juice, Berry Juice or Concentrates
Date Published: 05/27/2014

Desc:Blueberry Juice Concentrate (Frozen)
Problems: CARBENDAZIM (MBC);
30 A - - 01 Beverage Base, Berry
Date Published: 05/27/2014

Desc:Blueberry Juice Concentrate (Frozen)
Problems: CARBENDAZIM (MBC);

Sonderjansen BV
Date Published : 05/27/2014

Dorpsstraat 66 , Rosmalen, Noord-Brabant NETHERLANDS
20 D - - 02 Blueberry Juice, Berry Juice or Concentrates
Date Published: 05/27/2014

Desc:Blueberry Juice Concentrate (Frozen)
Problems: CARBENDAZIM (MBC);
30 A - - 01 Beverage Base, Berry
Date Published: 05/27/2014

Desc:Blueberry Juice Concentrate (Frozen)
Problems: CARBENDAZIM (MBC);

Van Bon Cold Stores Bv
Date Published : 09/16/2009

Zandstraat 141 , Beneden Leeuwen, NETHERLANDS
21 G - D 03 Cherry Fruit (Pit Fruit)
Date Published: 09/16/2009

Notes:7/12/04
Problems: FLUSILAZOLE;
21 G - T 03 Cherry Fruit (Pit Fruit)
Date Published: 09/16/2009

Notes:7/12/04
Problems: FLUSILAZOLE;

To: Miller, David[Miller.DavidJ@epa.gov]
Cc: Hrdy, David[Hrdy.David@epa.gov]
From: Councell, Terry
Sent: Thur 10/29/2015 5:10:27 PM
Subject: Glyphosate Project

David

Requires Consultation with FDA

Terry Councell

Total Diet Study Coordinator

Food and Drug Administration

Office of Analytics and Outreach

5100 Paint Branch Parkway

College Park, MD 20740

PH (240) 402 1180

BEYOND PESTICIDES
701 E Street, SE ▪ Washington DC 20003
202-543-5450 phone ▪ 202-543-4791 fax
info@beyondpesticides.org ▪ www.beyondpesticides.org

December 16, 2015

Jack Housenger
Director, Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Ave, NW
Washington DC 20460-0001

Re: Glyphosate testing on U.S. commodities.

Dear Mr. Housenger,

We are writing to express concern over the lack of routine residue testing for glyphosate in U.S. food commodities. Glyphosate is the most widely used herbicide in the U.S., used on millions of acres of crops grown across the country.[1] The U.S. Environmental Protection Agency (EPA) under the *Federal Insecticide Fungicide and Rodenticide Act* (FIFRA) as well as the *Food Quality Protection Act* (FQPA) has an obligation to safeguard human health from potential risks from glyphosate exposure, especially dietary exposures. Currently, there is no formal testing for residues of glyphosate on food commodities, and we are now asking EPA to ensure that there is routine U.S. testing for glyphosate on U.S. food.

The U.S. Department of Agriculture (USDA), Food and Drug Administration (FDA) and EPA all share some oversight on pesticide residues in food. From a regulatory standpoint, EPA sets pesticide limits in food through its risk assessment process under FIFRA. Currently, USDA's Pesticide Data Program (PDP) tests food commodities for pesticide residues. FQPA directs USDA to collect pesticide residue information on food highly consumed, particularly by infants and children.[2] The data gathered from this program helps EPA conduct and validate the accuracy of its dietary assessments for pesticides under FQPA. As a result, EPA can submit requests for data.

As mentioned, EPA utilizes residue data to estimate the exposure of the general population to pesticides in food. Residue data from PDP is therefore very useful in helping the agency set tolerance limits on food. Section 408(b)(2)(A)(i) of the *Federal Food Drug and Cosmetics Act*

[1] USEPA. 2006 and 2007 Market Estimates. Pesticides Industry Sales and Usage. http://www2.epa.gov/sites/production/files/2015-10/documents/market_estimates2007.pdf
[2] USDA. 2013. Pesticide Data Program. Annual Summary, Calendar Year 2011. Agricultural Marketing Service. Washington DC.

states that EPA can establish a tolerance for a pesticide chemical residue in or on food only if EPA determines that the tolerance is safe. "Safe" is then defined as a "reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures." Part of the tolerance setting process is the consideration of the amount of residue likely to remain on the food commodity. These limits must be based on human health data and should be informed by reliable real-world data. Without this, proper assessment of human health exposures can be left grossly underestimated, putting public health at risk. Currently the tolerances set for glyphosate range from 0.1ppm to 400ppm. On widely treated food commodities, glyphosate is set at 20ppm for soybean seed, 5ppm for corn, and 20ppm for canola seed.[3]

Currently, PDP is not testing for glyphosate residues on crops. In its last report, released in 2013, there was preliminary data for glyphosate on a small sample of soybeans.[4] 300 soybean samples were tested where 90 percent contained residues from 0.26ppm to 18.5ppm, barely under the tolerance limit of 20ppm. No residue testing has been done for corn.

Corn and soybean are the two most widely grown crops in the U.S., and the most treated with pesticides like glyphosate, due to the use of genetically engineered (GE) herbicide-tolerant strains. Further, corn and soybean make up the foundation of the American diet. Recently, EPA increased the glyphosate tolerances for other minor crops, like carrots and sweet potato, and many expect to see increases in glyphosate applications. Gathering residue information on glyphosate's presence on the foods Americans eat is critical to EPA's assessment of human health risks posed by this chemical. With the recent classification of glyphosate as a 'probable human carcinogen' by the World Health Organization's International Agency for Research on Cancer (IARC), and the impending release of glyphosate's registration review, many consumers are growing increasingly concerned about glyphosate exposures, making it more imperative that this information is made available.

We urge the agency to work with USDA to ensure that glyphosate becomes a routine part of the PDP's residue testing and that these tests are done more frequently. We would love the opportunity to talk further on this matter if needed. We look forward to your response.

Sincerely,

Nichelle Harriott
Science and Regulatory Director

[3] 40 CFR 180.364
[4] AMS. 2013. Pesticide Data Program. Annual Summary, Calendar Year 2011.

To: Councell, Terry[Terry.Councell@fda.hhs.gov]
From: Miller, David
Sent: Tue 1/5/2016 5:18:26 PM
Subject: RE: Glyphosate

(b) (5)

david

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, January 05, 2016 12:15 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate

Requires Consultation with FDA

Terry

From: Miller, David [mailto:Miller.DavidJ@epa.gov]
Sent: Tuesday, January 05, 2016 12:13 PM
To: Councell, Terry
Subject: RE: Glyphosate

(b) (5)

David.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, January 05, 2016 12:02 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: Glyphosate

David

Requires Consultation with FDA

Terry

To: Councell, Terry[Terry.Councell@fda.hhs.gov]
From: Miller, David
Sent: Tue 1/5/2016 5:12:49 PM
Subject: RE: Glyphosate

(b) (5)

David.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, January 05, 2016 12:02 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: Glyphosate

David

Requires Consultation with FDA

Terry

To: Councell, Terry[Terry.Councell@fda.hhs.gov]
From: Miller, David
Sent: Mon 1/4/2016 6:11:03 PM
Subject: RE: Glyphosate Discussion with Beyond Pesticides

Requires Consultation with FDA

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Monday, January 04, 2016 12:30 PM
To: Miller, David <Miller.DavidJ@epa.gov>
Subject: RE: Glyphosate Discussion with Beyond Pesticides

David

Requires Consultation with FDA

Terry

From: Miller, David [mailto:Miller.DavidJ@epa.gov]
Sent: Monday, January 04, 2016 11:34 AM
To: Councell, Terry
Subject: FW: Glyphosate Discussion with Beyond Pesticides

Hello Terry,

(b) (5)

David.

From: Miller, David
Sent: Monday, January 04, 2016 11:32 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>; Bloem, Thomas <Bloem.Thomas@epa.gov>; Steve Nako <Nako.Steve@epa.gov>
Subject: RE: Glyphosate Discussion with Beyond Pesticides

(b) (5)

David.

David.

From: Smith, Charles
Sent: Monday, January 04, 2016 11:21 AM
To: Miller, David <Miller.DavidJ@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>; Bloem, Thomas <Bloem.Thomas@epa.gov>
Subject: RE: Glyphosate Discussion with Beyond Pesticides

Dave,

(b) (5)

Thanks!

Charles " Billy" Smith

Branch Chief RAB1

Health Effects Division

Office of Pesticide Programs

703-305-0291

From: Miller, David
Sent: Monday, January 04, 2016 11:11 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>
Subject: FW: Glyphosate Discussion with Beyond Pesticides

(b) (5)

David.

To: Councell, Terry[Terry.Councell@fda.hhs.gov]
From: Miller, David
Sent: Mon 1/4/2016 4:33:32 PM
Subject: FW: Glyphosate Discussion with Beyond Pesticides
EPA glyphosate testing letter2015.pdf

Hello Terry,

(b) (5)

David.

From: Miller, David
Sent: Monday, January 04, 2016 11:32 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>; Bloem, Thomas <Bloem.Thomas@epa.gov>; Steve Nako <Nako.Steve@epa.gov>
Subject: RE: Glyphosate Discussion with Beyond Pesticides

(b) (5)

David.

David.

From: Smith, Charles
Sent: Monday, January 04, 2016 11:21 AM
To: Miller, David <Miller.DavidJ@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>; Bloem, Thomas <Bloem.Thomas@epa.gov>
Subject: RE: Glyphosate Discussion with Beyond Pesticides

Dave,

(b) (5)

Thanks!

Charles " Billy" Smith

Branch Chief RAB1

Health Effects Division

Office of Pesticide Programs

703-305-0291

From: Miller, David
Sent: Monday, January 04, 2016 11:11 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Hrdy, David <Hrdy.David@epa.gov>
Subject: FW: Glyphosate Discussion with Beyond Pesticides

(b) (5)

David.

BEYOND PESTICIDES
701 E Street, SE ▪ Washington DC 20003
202-543-5450 phone ▪ 202-543-4791 fax
info@beyondpesticides.org ▪ www.beyondpesticides.org

December 16, 2015

Jack Housenger
Director, Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Ave, NW
Washington DC 20460-0001

Re: Glyphosate testing on U.S. commodities.

Dear Mr. Housenger,

We are writing to express concern over the lack of routine residue testing for glyphosate in U.S. food commodities. Glyphosate is the most widely used herbicide in the U.S., used on millions of acres of crops grown across the country.[1] The U.S. Environmental Protection Agency (EPA) under the *Federal Insecticide Fungicide and Rodenticide Act* (FIFRA) as well as the *Food Quality Protection Act* (FQPA) has an obligation to safeguard human health from potential risks from glyphosate exposure, especially dietary exposures. Currently, there is no formal testing for residues of glyphosate on food commodities, and we are now asking EPA to ensure that there is routine U.S. testing for glyphosate on U.S. food.

The U.S. Department of Agriculture (USDA), Food and Drug Administration (FDA) and EPA all share some oversight on pesticide residues in food. From a regulatory standpoint, EPA sets pesticide limits in food through its risk assessment process under FIFRA. Currently, USDA's Pesticide Data Program (PDP) tests food commodities for pesticide residues. FQPA directs USDA to collect pesticide residue information on food highly consumed, particularly by infants and children.[2] The data gathered from this program helps EPA conduct and validate the accuracy of its dietary assessments for pesticides under FQPA. As a result, EPA can submit requests for data.

As mentioned, EPA utilizes residue data to estimate the exposure of the general population to pesticides in food. Residue data from PDP is therefore very useful in helping the agency set tolerance limits on food. Section 408(b)(2)(A)(i) of the *Federal Food Drug and Cosmetics Act*

[1] USEPA. 2006 and 2007 Market Estimates. Pesticides Industry Sales and Usage. http://www2.epa.gov/sites/production/files/2015-10/documents/market_estimates2007.pdf
[2] USDA. 2013. Pesticide Data Program. Annual Summary, Calendar Year 2011. Agricultural Marketing Service. Washington DC.

states that EPA can establish a tolerance for a pesticide chemical residue in or on food only if EPA determines that the tolerance is safe. "Safe" is then defined as a "reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures." Part of the tolerance setting process is the consideration of the amount of residue likely to remain on the food commodity. These limits must be based on human health data and should be informed by reliable real-world data. Without this, proper assessment of human health exposures can be left grossly underestimated, putting public health at risk. Currently the tolerances set for glyphosate range from 0.1ppm to 400ppm. On widely treated food commodities, glyphosate is set at 20ppm for soybean seed, 5ppm for corn, and 20ppm for canola seed.[3]

Currently, PDP is not testing for glyphosate residues on crops. In its last report, released in 2013, there was preliminary data for glyphosate on a small sample of soybeans.[4] 300 soybean samples were tested where 90 percent contained residues from 0.26ppm to 18.5ppm, barely under the tolerance limit of 20ppm. No residue testing has been done for corn.

Corn and soybean are the two most widely grown crops in the U.S., and the most treated with pesticides like glyphosate, due to the use of genetically engineered (GE) herbicide-tolerant strains. Further, corn and soybean make up the foundation of the American diet. Recently, EPA increased the glyphosate tolerances for other minor crops, like carrots and sweet potato, and many expect to see increases in glyphosate applications. Gathering residue information on glyphosate's presence on the foods Americans eat is critical to EPA's assessment of human health risks posed by this chemical. With the recent classification of glyphosate as a 'probable human carcinogen' by the World Health Organization's International Agency for Research on Cancer (IARC), and the impending release of glyphosate's registration review, many consumers are growing increasingly concerned about glyphosate exposures, making it more imperative that this information is made available.

We urge the agency to work with USDA to ensure that glyphosate becomes a routine part of the PDP's residue testing and that these tests are done more frequently. We would love the opportunity to talk further on this matter if needed. We look forward to your response.

Sincerely,

Nichelle Harriott
Science and Regulatory Director

[3] 40 CFR 180.364
[4] AMS. 2013. Pesticide Data Program. Annual Summary, Calendar Year 2011.

To: Haynes, Diana - AMS[Diana.Haynes@ams.usda.gov]; Smith, Charles[Smith.Charles@epa.gov]
Cc: Hrdy, David[Hrdy.David@epa.gov]; Vogel, Dana[Vogel.Dana@epa.gov]; Jordan, William[Jordan.William@epa.gov]; Councell, Terry[Terry.Councell@fda.hhs.gov]; Crowley, Matthew[Crowley.Matthew@epa.gov]; Nako, Steve[Nako.Steve@epa.gov]
From: Miller, David
Sent: Tue 11/10/2015 8:48:08 PM
Subject: FW: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

FYI – the good news in the link that Terry provided is that of the following tested commodities, it was only found in the (unprocessed) honey

- 69 samples of honey
- 26 samples of pancake and corn syrup
- 28 samples of soy sauce
- 11 samples of soy milk
- 20 samples of tofu

(b) (5)

David.

From: Councell, Terry [mailto:Terry.Councell@fda.hhs.gov]
Sent: Tuesday, November 10, 2015 2:31 PM
To: Miller, David <Miller.DavidJ@epa.gov>; Hrdy, David <Hrdy.David@epa.gov>
Subject: FW: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

FYI

From: Chamkasem, Narong
Sent: Tuesday, November 10, 2015 2:24 PM
To: Sack, Chris A; Councell, Terry; Yeung, Andrew; Podhorniak.Lynda@epa.gov; Islam, Mohammed R
Cc: Harmon, Tiffany M
Subject: Researchers Discover Glyphosate Herbicide in Honey, Soy Sauce | Natural Society

http://naturalsociety.com/researchers-discover-glyphosate-herbicide-honey-soy-sauce/